Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Fisheries and Oceans Pêches et Océans

Canada

Scientific Excellence • Resource Protection & Conservation • Benefits for Canadians

Excellence scientifique • Protection et conservation des ressources • Bénéfices aux Canadiens

The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79.

Editorial Policy

The *Journal* publishes original research articles, critical reviews, perspectives (essays of opinion or hypothesis), and comments. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or syntheses, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmation state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

It would assist the Editors if prospective authors identified briefly by covering letter (a) aspects of their papers that meet the foregoing objectives, (b) potential referees, and (c) other manuscripts contemplated or in press containing the same or similar information.

Submissions in English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company.

A charge is made for manuscripts published. When submitting a manuscript, an author must state that the charge will be accepted.

Guides

Guides for authors and typists appear in the first issue of each volume and are available free from the *Journal*.

Subscriptions

The *Journal* is issued monthly to form annual volumes of twelve numbers plus Supplements. Orders and inquiries regarding subscriptions must be sent directly to Supply and Services Canada, Ottawa, Ontario, Canada K1A 0S9. Prepayment is required in Canadian funds to the order of "The Receiver General for Canada."

Microfilm

Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

Printed in Canada by: The Runge Press Limited, Ottawa

Imprimé au Canada par: The Runge Press Limited, Ottawa

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979.

Politique de rédaction

Le *Journal* publie des articles fondés sur une recherche originale, des critiques, des essais portant sur une opinion ou une hypothèse (perspectives) et des commentaires. Les textes peuvent avoir trait aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influent sur les systèmes de production aquatique. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. Ils doivent clairement démontrer qu'ils contribuent aux connaissances en faisant plus que corroborer des faits. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles.

Les auteurs éventuels aideraient les rédacteurs s'ils identifiaient brièvement, dans une lettre d'accompagnement a) les aspects de leurs textes qui répondent particulièrement aux objectifs indiqués ci-dessus, b) des arbitres possibles et c) d'autres manuscrits envisagés ou sous presse, dont la teneur est identique ou se rapproche de celui qui est soumis.

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le *Journal* décline toute responsabilité quant aux énoncés des contributeurs. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

Les manuscrits acceptés sont sujets à des frais de publication. Quand un auteur présente un texte, il doit préciser que ces frais seront acceptés. On trouvera dans une récente livraison du *Journal* les taux par page en vigueur.

Guides

Le guide des auteurs et celui des secrétaires d'auteurs paraissent dans la première livraison de chaque volume et sont offerts gratuitement par le *Journal*.

Abonnement

Le *Journal* paraît tous les mois, formant ainsi un volume de douze numéros, et offre en plus jusqu'à deux suppléments par année. On doit faire parvenir les commandes et demandes de renseignements concernant les abonnements à Approvisionnements et Services Canada, Ottawa (Ontario), Canada K1A 0S9. Les paiements doivent être faits à l'avance en devises canadiennes, à l'ordre du Receveur général du Canada.

Microfilms

On peut acheter sur microfilms de 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, É.-U.

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 47, No. 11, November 1990 **Volume 47, nº 11, novembre 1990**

Scientific Publications/Publications scientifiques

D. G. Cook, Ph.D. — *Editor/Rédacteur*
J. Camp/G. J. Neville/C. Beaulac/ M. Milne — *Editorial and Publishing Services/Services de rédaction et d'édition*
P. E. K. Symons, Ph.D. — *Consulting Editor/Rédacteur-conseil (Victoria, B.C.)*

Associate Editors/Rédacteurs associés

J. R. M. Kelso, Ph.D.	*Department of Fisheries and Oceans, Sault Ste. Marie*
C. D. Levings, Ph.D.	*Department of Fisheries and Oceans, West Vancouver*
J. D. Neilson, Ph.D.	*Department of Fisheries and Oceans, St. Andrews*
P. Schwinghamer, Ph.D.	*Department of Fisheries and Oceans, St. John's*
N. H. F. Watson, Ph.D.	*Department of Fisheries and Oceans, Halifax*
D. J. Noakes, Ph.D.	*Department of Fisheries and Oceans, Nanaimo*

Editorial Board/Conseil de rédaction

P. V. Hodson	*Department of Fisheries and Oceans, Mont-Joli*	(1987–1990)
D. R. S. Lean	*Department of the Environment, Burlington*	(1987–1990)
R. M. Peterman	*Simon Fraser University*	(1987–1990)
R. A. Stein	*Ohio State University*	(1987–1990)
S. J. Smith	*Department of Fisheries and Oceans, Dartmouth*	(1988–1991)
J. C. Roff	*University of Guelph*	(1990–1992)
L. M. Dickie	*Department of Fisheries and Oceans, Dartmouth*	(1990–1992)

Editorial Office:

Department of Fisheries and Oceans
Communications Directorate
Scientific Publications
200 Kent Street, 14th Floor
Ottawa, Canada K1A 0E6
Telephone (613) 993–2209
FAX (613) 990-1866

Bureau de rédaction :

Ministère des Pêches et des Océans
Direction générale des communications
Publications scientifiques
200, rue Kent, 14e étage
Ottawa, Canada K1A 0E6
Numéro de téléphone (613) 993–2209
FAX (613) 990-1866

The *Journal* is abstracted or indexed in:/Le *Journal* est résumé ou signalé dans :

Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, Oceanic Abstracts, and Science Citation Index

Distribution of Shrimp (*Pandalus borealis*) Larvae and Hydrographic Pattern in the Northern Gulf of St. Lawrence[1]

Patrick Ouellet[2] and Denis Lefaivre

Department of Fisheries and Oceans, Maurice Lamontagne Institute, 850 route de la Mer, Mont Joli, Québec G5H 3Z4 Canada

and Vladimir Koutitonsky

Institut National de la Recherche Scientifique, 310 des Ursulines, Rimouski, Québec G5L 3A1 Canada

Ouellet, P., D. Lefaivre, and V. Koutitonsky. 1990. Distribution of shrimp (*Pandalus borealis*) larvae and hydrographic pattern in the northern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 47: 2068–2078.

The large-scale spatial distribution of northern shrimp (*Pandalus borealis*) larvae was analyzed in the Gulf of St. Lawrence during the spring of 1986 and 1987. Although sites of emergence are related to the principal aggregation areas of adult shrimp, it appears that the currently fished aggregations may not represent isolated populations. The uniformity of developmental stages and the wide-spread distribution of the first larval stage were consistent with the hypothesis of synchronous larval emergence among the northern Gulf shrimp. The structured pattern of larval spatial distribution was not correlated with the hydrographic structure within the different sectors of the Gulf (Mantel test; $P > 0.05$). Spatial correlograms showed that the highly structured spatial distribution pattern of stage I larvae evolved into a spatially random pattern by the time stage III larvae had developed. We suggest that biological or ecological dynamic processes such as larval mortality and development rates are more important than the hydrodynamics of the sectors in determining the structure of larval shrimp distribution. Consequently, on a seasonal basis, the distribution of larval shrimp groups cannot be inferred from a study of the hydrographic pattern within the sectors at the scale of our analysis.

La distribution spatiale à grande échelle des stades larvaires de la crevette nordique (*Pandalus borealis*) a été analysée dans le golfe du Saint-Laurent au printemps de 1986 et 1987. En dépit des sites d'émergence correspondant aux principales zones d'agrégation des crevettes adultes, il ne semble pas que les groupes exploités actuellement représentent des populations isolées. L'uniformité des stades de développement et l'étendue de la dispersion du premier stade larvaire supportent l'idée d'une émergence synchronisée des larves pour les crevettes du nord du Golfe. La structure spatiale de la distribution larvaire n'était pas corrélée à la structure hydrographique des divers secteurs du Golfe (test de Mantel; $P > 0.05$). Les corrélogrammes spatiaux montrent que la distribution spatiale très structurée des larves de stade I a évolué en une distribution spatiale aléatoire au moment où les stades III sont apparues dans la population. Le fait est interprété comme indicateur de l'importance des facteurs biologiques ou écologiques de la survie larvaire et du développement, par rapport aux processus hydrodynamiques, pour expliquer la structure de la distribution spatiale des larves de crevette. En conséquence, sur une base saisonnière, il n'est pas possible de déduire la distribution des groupes larvaires de crevette à partir de l'analyse hydrographique des secteurs à l'échelle étudiée.

Received July 19,1989
Accepted May 24, 1990
(JA236)

Reçu le 19 juillet 1989
Accepté le 24 mai 1990

Shrimp *Pandalus borealis* is a valuable fishery in the Gulf of St. Lawrence, with approximately 10 000 t landed annually since 1980 (Savard et al 1988). The results of scientific surveys in the early seventies (S. Labonté, Department of Fisheries and Oceans, MLI, 850 route de la Mer, Mont-Joli, Québec G5L 3A1, pers. comm.), and recent analyses of the geographic distribution of commercial catch data have led to the management of the fishery based on three principal shrimp aggregations found in the northern Gulf: (1) northwest, (2) north Anticosti Island, and (3) the northeast sectors (Fig. 1). The nature of these aggregations as independent biological populations (i.e. isolated reproductive units) is an important issue in determining an appropriate management strategy. Biomass and the demographic structure of the different aggregations are assessed annually using commercial catch data and scientific trawl surveys (Savard et al 1988). Recently, demograhic analyses have revealed important variations in the proportion of length-classes (age-groups) among sectors and years (Simard and Savard 1990). Variability in the abundance of the age-groups is consistent with the hypothesis that recruitment processes within sectors are responsible for the abundance variations observed among sectors.

Adult shrimp spawn in early fall in the Gulf of St. Lawrence. The egg mass is carried by the female during the winter months and in spring the berried females move toward shallower water where the first pelagic larval stage is released into the water column. The first two zoeal stages are concentrated in the upper mixed layer (average depth of 30 m) above the permanent thermocline, coincident with the depth of the sub-surface chlorophyll *a* biomass maximum (P. Ouellet unpubl. data). The first pelagic larval stage is a fully developed zoea capable of swim-

[1]Contribution to Circulation, Oceanography, and Hydrography of Jacques-Cartier passage [COHJAC] program.

[2]Present address: Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, B3H 4J1 Canada.

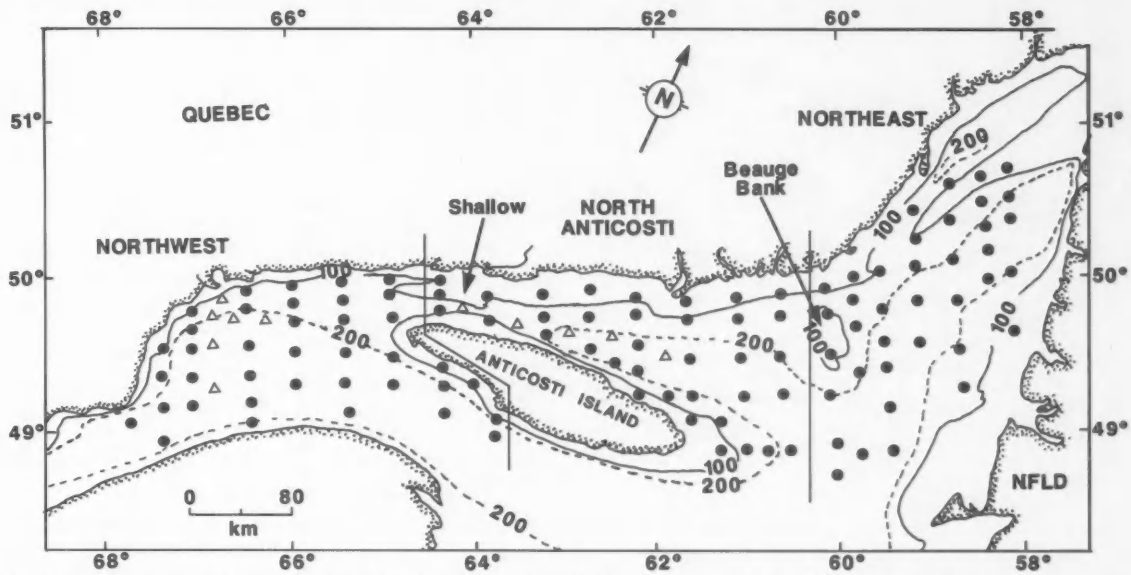

FIG. 1. Bathymetric chart of the geographic subdivisions of the northern Gulf of St. Lawrence and sampling grid for the 1986 and 1987 surveys: (●) permanent stations; (△) supplementary stations sampled in May 1987.

ming and active feeding (Stickney and Perkins 1981). Pelagic development entails five stages and normally terminates with settlement during the sixth (megalopa) stage (Berkeley 1931). The duration of development is a function of temperature and feeding conditions and can last 3 mo at 5°C (Shumway et al 1985). The pelagic early life history suggests that hydrographic variations play a major role in determining the extent of larval abundance distribution.

In discussing the mechanisms regulating abundance in marine fish populations, Sinclair and Iles (1988) proposed that location of sexual reproduction and subsequent discrete egg and larvae distributions in relation to physical oceanographic features provide the ultimate control of geographical population identification, persistence, and abundance. In the context of this proposition, this paper examines the hypothesis that local production of larval groups coupled with specific hydrographic characteristics of the different sectors of the Gulf will result in the effective isolation of larvae among sectors and hence the resultant discrimination of adult population units.

We report here the first results of a research program begun in 1986 to obtain information on shrimp, *P. borealis*, larvae in the Gulf of St. Lawrence. The principal objective of the program is to identify the major processes affecting recruitment in the Gulf shrimp. In the first part of the program our specific objectives were: (1) to identify the zones of larval release and to determine the spatial distribution of the larvae in the northern Gulf, and (2) to examine the relation between the hydrographic patterns of the sectors and the distribution of the early larval stages.

Materials and Methods

Surveys

A station grid was designed to obtain representative samples from the entire northern Gulf of St. Lawrence (Fig. 1). A complete survey consisted of 112 sampling stations spaced at an average of 15 km intervals on north–south transects each spaced at 40 km. The station grid was sampled twice in 1986 (30 April–12 May, and 26 May–9 June) and once in 1987 (9 May–19 May), hereafter referred as May 1986, June 1986, and May 1987 sampling periods. Zooplankton were sampled at each station using one double-oblique tow from 0 to 150 m (depth permitting) with a MARMAP-Bongo frame (Posgay and Marak 1980) fitted with two 333 μm-mesh nets and General Oceanics flowmeters. Temperature (expendable bathythermograph [XBT]), or temperature and salinity profiles (Guildline conductivity, temperature, and depth probe [CTD]) were obtained concurrently with the zooplankton sampling.

All zooplankton collections were preserved immediately in a 4% formalin and seawater solution. One sample from the Bongo net was sorted for shrimp larvae when the number (N) of larvae (all stages) was ≥ 50; if $N < 50$ the second sample was sorted and the results combined for the analyses. The larvae of *Pandalus borealis* were identified following the descriptions of Berkeley (1931), Pike and Williamson (1964), and Haynes (1979). To distinguish the larvae of *P. borealis* from *P. montagui* we employed morphometric data as *P. montagui* is smaller than *P. borealis* at any stage. However, identification was unsure when the total length of the specimen could not be obtained. In such cases, and if the larvae could be staged, the two species were distinguished by the number of setae on the antenna scale as follows:

	Zoea I	Zoea II	Zoea III
Pandalus borealis	15–19	25–28	> 30
Pandalus montagui	< 10	15–20	< 30

The larvae were counted, staged, and measured for carapace (posterior edge of orbit to mid-dorsal posterior margin of carapace) and total length (tip of rostrum to end of telson). In 1986 all measurements were made with an optical micrometer

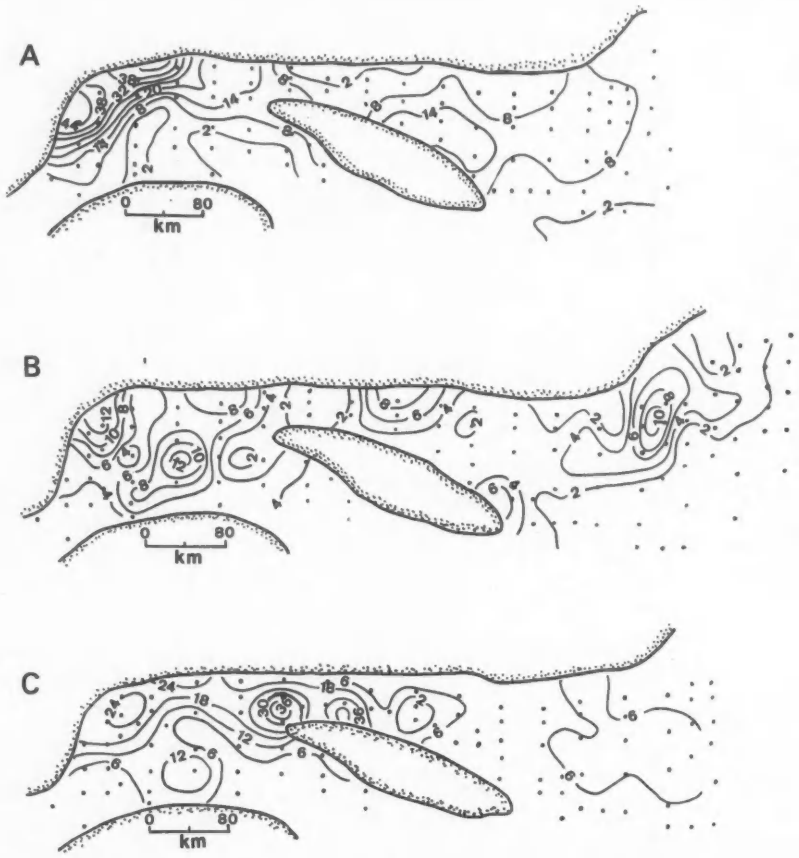

FIG. 2. Spatial distribution of *P. borealis* larvae (larvae·m^{-2}) in the northern Gulf of St. Lawrence (contours lines): (A) stage I — May 1986; (B) stage II — June 1986; and (C) stage I — May 1987. Sampling stations are denoted by (●).

TABLE 1a. Proportion (%) of *P. borealis* larvae in each developmental stage, in each sector, during the sampling periods in the Northern Gulf of St. Lawrence.

Sector	Stage	Period		
		May 1986	June 1986	May 1987
Northwest	I	100.0	15.0	96.4
	II	—	81.0	3.6
	III	—	4.0	—
N-Anticosti	I	100.0	1.2	94.0
	II	—	86.8	6.0
	III	—	12.0	—
Northeast	I	—	1.5	—
	II	—	84.0	—
	III	—	15.5	—

TABLE 1b. Approximate area (km^2) of each sector, shrimp larvae average concentration (larvae·m^{-2}) and SD for each stage and for each sampling period, in the northern Gulf of St. Lawrence.

Period	Stage	Northwest (3.2×10^4)	N-Anticosti (5.3×10^4)	Northeast (3.5×10^4)
May 1986	I	15.09 ± 17.3	7.96 ± 7.1	—
		N = 38	N = 39	
June 1986	I	1.06 ± 1.91	0.04 ± 0.16	0.04 ± 0.13
	II	5.78 ± 4.75	3.11 ± 3.01	2.45 ± 3.54
	III	0.27 ± 0.41	0.42 ± 0.66	0.42 ± 0.62
		N = 38	N = 33	N = 37
May 1987	I	13.16 ± 12.7	9.68 ± 14.1	—
	II	0.48 ± 0.8	0.36 ± 0.53	—
		N = 46	N = 39	

mounted on a dissecting lens while in 1987 the lengths were obtained electronically by processing digitized larval images. Abundance estimates were standardized to number per square metre of sea surface to the maximum sampling depth (Smith and Richardson 1977). Larval distributions were plotted using a kriging algorithm for objective interpolation of values among stations (Golden Software, Golden, CO).

Analysis of Hydrographic Data

Temperature profiles from XBT probes were available for 26 of the 39 stations in the north Anticosti sector during the first

survey in 1986. During the second survey, only nine out of 38 stations in the northwest sector were covered, but temperature and salinity profiles were available for the north Anticosti sector and for 26 out of 37 stations in the northeast sector. In 1987, CTD measurements were taken at all stations.

The horizontal distribution of average temperature and salinity in the upper mixed layer (between 0 and 10 m, and 20 and 30 m), and the vertical distribution of isopycnals over cross-sections were computed to resolve the basic hydrodynamic structure of the region during the sampling periods. A clustering technique was used to provide a numerical description of the spatial heterogeneity of the water masses for statistical analysis. To achieve this, each temperature (T) and salinity (S) profile was divided into six vertical strata (i) at 10 m intervals over the first 60 m of the water column and at 20 m intervals below 60 m. The different spacing of the intervals reflects the greater heterogeneity of the mixed layer relative to the intermediate (below 60 m) and deep layers in the Gulf. The average temperature (T_i) and salinity (S_i) pairs, within each stratum, were used as coordinates to position the station strata on a T-S diagram compiled from data for the entire Gulf region (D. Lefaivre, unpubl. data). Numeric codes for the water type, determined by the location of the strata coordinates on the T-S diagram, were then attributed to the station strata. Thus, the temperature and salinity profile at each station was converted into a vector of a finite number of elements.

A numeric similarity value (i.e. distance) for every station pair was then estimated for each sampling period using the simple matching coefficient (SM) for semi-quantitative data (Sneath and Sokal 1973). The unweighed pair average (UPGMA) clustering protocol in the NTSYS-pc statistical package (Rohlf 1987) was then applied to the similarity matrix. A similar procedure was used for temperature data (mean temperature within stratum) from the XBT probes used in May 1986 except that the SM coefficient was replaced by the Euclidean distance recommended for continuous data (Legendre and Legendre 1984).

Spatial Patterns

Spatial autocorrelation procedures and the Mantel test of matrix correspondence (Sokal and Oden 1978; Sokal and Thomson 1987) were used to quantify the spatial patterns in the distribution of *P. borealis* larvae in each sector and for each sampling period. This permitted comparisons among sectors and an assessment of the relationship between the distribution of the larvae and the hydrographic pattern. Spatial autocorrelation is a measure of the dependence of variable 'x_i' on the values of 'x' at neighbouring stations, where the "neighbourhood" of the stations is defined by different geographical distance-classes among stations. The distance-classes for which the correlation remains significant and positive indicates the length of a patch. A progression from positive to negative correlations along a correlogram is indicative of a distribution gradient (Sokal and Oden 1978). Moran's I was chosen as the univariate equivalent of the standard correlation coefficient.

The relation between Moran's I and geographic distance was determined for each sector and sampling period. The significance of each correlogram was tested by the Bonferroni technique for small class (< 10) correlograms (Oden 1984). The first class intervals in the correlograms were defined by the maximum distance between stations on a transect (0–20 km for the first class), and the maximum distance between transects (20–40 km for the second class). The remaining classes were

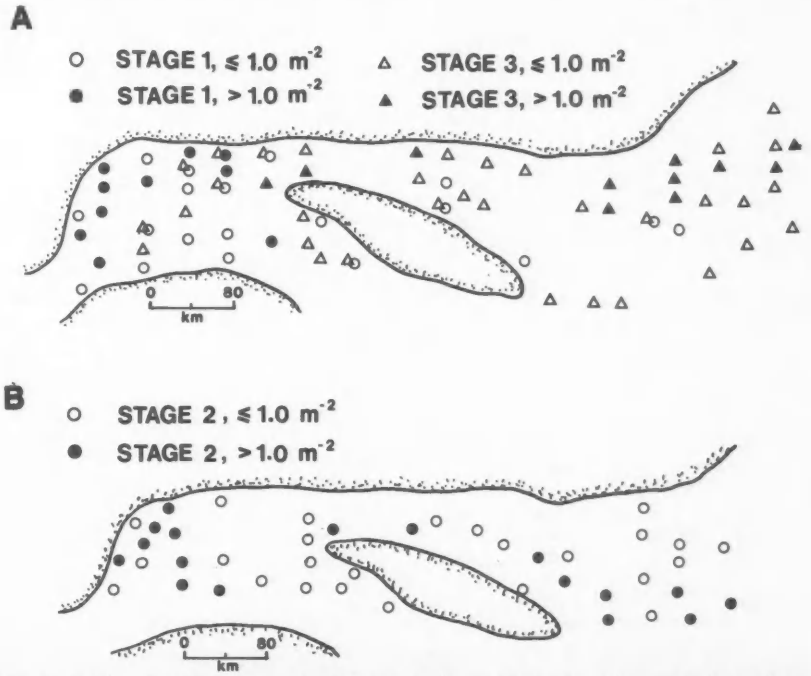

FIG. 3. Position of stations where more than one developmental stage was observed in the northern Gulf of St. Lawrence: (A) June 1986; and (B) May 1987. The figure should be compared with Fig. 2.

determined to obtain a uniform frequency distribution of the geographical distances between the intervals.

The multivariate equivalent of univariate spatial autocorrelation was obtained from the general Mantel test of correspondence between two matrices (Legendre 1987; Sokal and Thomson 1987). Given matrix **A** of dissimilarity indices between every pair in a set of objects (stations) and matrix **B** describing the relation between the same set for a second and independent variable, the test statistic **Z** is calculated by the summation of the element by element product of the two matrices:

$$\mathbf{Z} = \Sigma_i \Sigma_j \mathbf{A}_{ij}\mathbf{B}_{ij} \qquad \text{for } i \neq j$$

The statistical significance of **Z** is estimated by calculating a family of **Z** values by random permutations of the rows and columns of one of the matrices assuming no linear relationship between the elements of the two matrices (Sokal and Thomson 1987). This test was used to assess the presence of spatial gradients in hydrographic variability; defined in the matrix computed from the *T-S* profiles [Hyd] in relation to a matrix of geographical distance [Geo] among stations. Similarly, to measure the spatial dependence of the biological variate, a matrix [Bio] describing the relation between stations was computed:

$$\mathbf{B} = |x_1 - x_2| / R$$

where x_n is larval abundance at stations, and R is the maximum difference between stations (range) in the data set. The relation between the biological and the hydrographic variability was assessed by computing the Mantel statistic between [Hyd] and [Bio].

Results

Distribution of *P. borealis* Larvae

Only zoea I larvae were caught in May 1986 (Table 1a), indicating that sampling coincided with larval release in all sectors. The average larval abundance was highest in the northwest sector (Table 1b), with the maximum concentrations (>44 larvae·m^{-2}) located along the coast (Fig. 2A). This region of high abundance is most easily interpreted as the site of larval release for that part of the Gulf. In the north Anticosti sector, zoea I larvae were observed in lower concentrations relative to the northwest sector (Table 1b). The data suggest that larvae emerged on the north side of the Island with evidence of subsequent eastward advection. Ice cover prevented sampling on the northeast sector in May 1986.

In June 1986, the larval population was composed mostly (>80%) of zoea II larvae over all sectors (Table 1a). In the northwest sector, the large patch of zoea I seen earlier had evolved into a complex pattern of dispersed zoea II larval

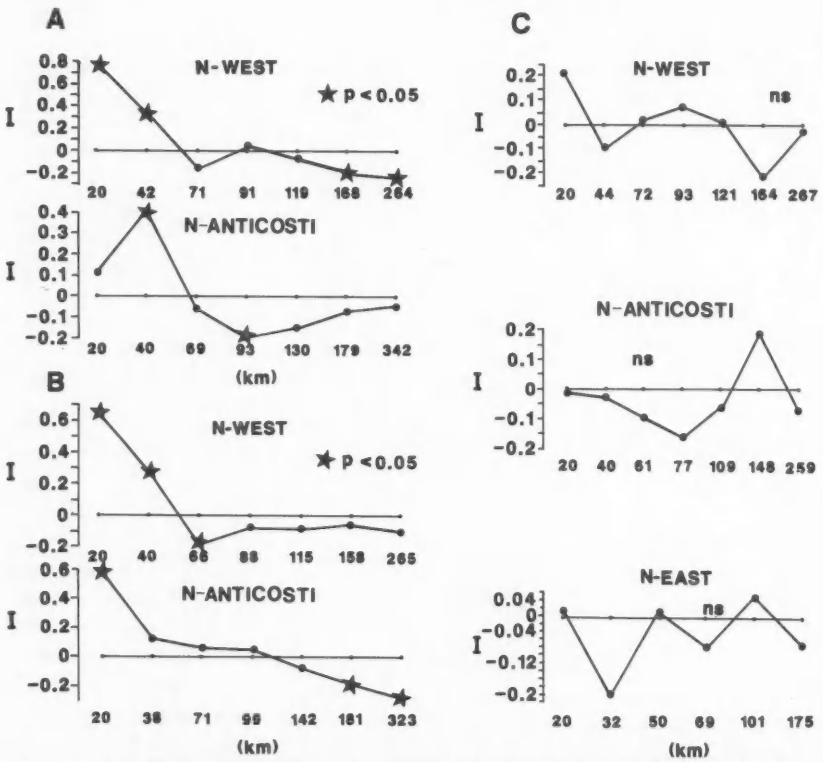

FIG. 4. Spatial correlograms illustrating the dispersion of *P. borealis* larvae in the northern Gulf of St. Lawrence: (A) May 1986—stage I, northwest and north Anticosti sectors; (B) May 1987—stage I, northwest and north Anticosti sectors; and (C) June 1986—stage II, northwest, north Anticosti, and northeast sectors.

TABLE 2. Average total length (tl (mm)), carapace length (cl (mm)) and SD of shrimp larvae for each sector and sampling period.

Period	Stage		Northwest	N-Anticosti	Northeast
May 1986	I	tl	6.404 ± 0.29 N = 846	6.469 ± 0.31 N = 910	—
		cl	1.292 ± 0.07 N = 846	1.288 ± 0.06 N = 910	—
June 1986	I	tl	6.587 ± 0.37 N = 57	6.64 ± 1.28 N = 5	6.60 ± 0.44 N = 3
		cl	1.303 ± 0.06 N = 57	1.28 ± 0.08 N = 5	1.30 ± 0.01 N = 3
	II	tl	8.113 ± 0.42 N = 418	8.161 ± 0.37 N = 224	8.15 ± 0.41 N = 148
		cl	1.926 ± 0.05 N = 418	1.918 ± 0.06 N = 224	1.907 ± 0.05 N = 148
	III	tl	9.87 ± 0.36 N = 20	9.87 ± 0.22 N = 27	10.0 ± 0.12 N = 28
		cl	2.39 ± 0.07 N = 20	2.37 ± 0.12 N = 27	2.40 ± 0.08 N = 28
May 1987[a]	I	tl	7.01 ± 0.5 N = 505	6.93 ± 0.36 N = 479	—
		cl	1.23 ± 0.14 N = 505	1.21 ± 0.11 N = 479	—
	II	tl	8.06 ± 0.49 N = 13	8.41 ± 0.38 N = 26	—
		cl	1.5 ± 0.19 N = 13	1.62 ± 0.16 N = 26	—

[a]The different techniques used for measuring the larvae between 1986 and 1987 may have created the inconsistency observed in average size of the stage I larvae. Hence, one should not compare the average total length between sampling periods.

groups (Fig. 2B). However, high zoea II larval abundances occurred along the northwest coast as previously observed with zoea I larvae. The coincidence at some stations of zoea I, II, and III larvae is indicative of larval persistence in certain areas (Fig. 3A).The average larval abundance was greatly reduced relative to May (Table 1b), indicating that either dispersion and/or mortality occurred during the sampling interval.

Larval maxima were observed along the Québec northshore in the north Anticosti sector, and at the southeastern end of the Island (Fig. 2B). High larval abundance was observed over Beaugé Bank in the northeast sector (Fig. 2B). The coincidence at some stations of zoea I, II, and III larvae again could be interpreted as local maintenance of cohorts (Fig. 3A). The dominance of zoea II larvae (84%) in the northeast sector was indicative of earlier under-ice larval release.

In May 1987 zoea I larvae again dominated (95%) in all sectors (Table 1a). Highest larval concentrations (>24 larvae·m^{-2}) occurred along the northwest coast (Fig. 2C), while abundance decreased markedly toward the southeastern edge of the sector. Larvae over the shallow region were indicative either of emergence-site displacement relative to May 1986, or of larval transport from the northwest to the north Anticosti sector. Abundance was greatest (>30 larvae·m^{-2}) at the western end of the north Anticosti sector, and the population appeared to be more or less contiguous with the northwest larval groups (Fig. 2C). Relatively low larval abundances were observed at the eastern limit of the sector (Table 1b, Fig. 2C). As in 1986, zoea I and II larvae were coincident at some stations, again suggesting the maintenance of larvae in discrete areas (Fig. 3B).

Spatial Autocorrelation

Spatial correlograms for the May periods (stage I larvae) demonstrated gradients of dispersion in the western and north Anticosti sectors (Fig 4A, 4B). The general pattern shows that stations closer together (<40 km) were more likely to have similar abundance values. In the northwest sector, the correlograms revealed greater heterogeneity in the May 1987 distribution relative to May 1986. Significant correlations passed from positive to negative between 40 km and 66 km, indicating the relatively small scale variation in abundance (Fig. 4B). The correlograms for the north Anticosti sector indicate inter-annual variability in larval distribution (Fig. 4A, 4B). In May 1986, a positive correlation at 40 km and a decay to a negative value at about 90 km are consistent with the observed larval aggregation along the north coast of the Island. The May 1987 correlogram reflects the distribution gradient from the west end of the sector toward the east.

In contrast to the correlograms for the earlier sampling periods, the correlograms for the stage II larvae in all sectors in June 1986 were not significant (Fig. 4C). These results indicate a random pattern of variation in larval abundance among stations.

Larval Length Distribution

No differences were observed between the average total length and carapace length within the larval stages among sectors during a sampling period (t-test; $P < 0.001$; Table 2). The homogeneity of larval stages observed in the sectors during each sampling period (Table 1a) illustrates the temporal uniformity of the reproductive pattern of the Gulf shrimp.

Hydrographic Patterns

In June 1986, three different hydrograhpic patterns were apparent in the combined north Anticosti and northeast sectors

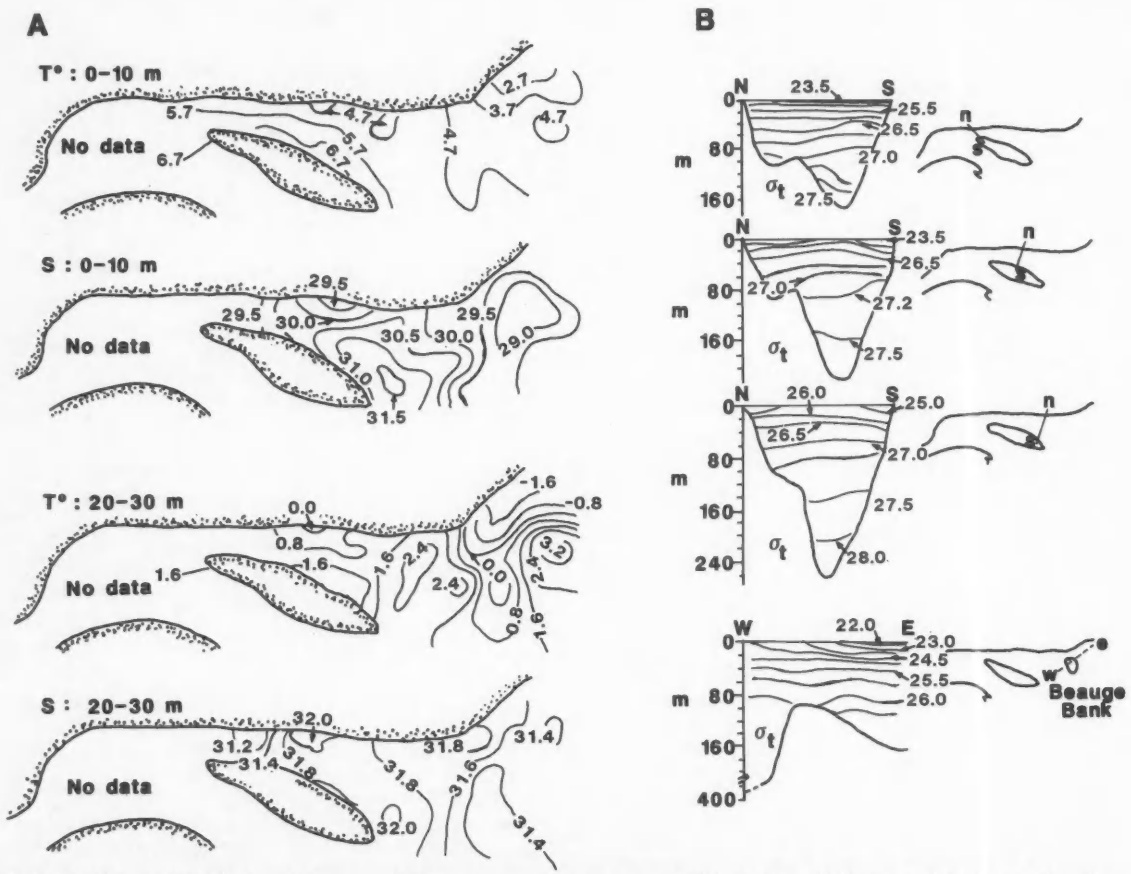

FIG. 5. (A) Horizontal distribution of the upper mixed-layer average temperature and salinity in June 1986 in the north Anticosti and northeast sectors of the northern Gulf of St Lawrence. (B) Isopycnal distribution at cross-sections in the north Anticosti sector.

(Fig. 5A). The area between the Island and the Québec northshore was characterized by (1) a weak salinity front over the shallow area at the west end of the Island, and (2) a gradient in water density in the upper 10 m of the water column with colder and fresher water along the Québec northshore and warmer, more saline water along the north side of the Island (Fig. 5A). The area included between the east end of Anticosti Island and Beaugé Bank was characterized by a gyre-like distribution of the temperature and salinity contours (Fig. 5A). Finally, a marked temperature and salinity front delimited the western edge of Beaugé Bank (Fig. 5A, 5B) and gyre-like circulation was observed over the Bank itself.

The isopycnal cross-sections indicated a relatively well-mixed surface layer ovér the shallow area (Fig. 5B). Farther east, the data indicated surface coastal circulation with a westward surface current along the Québec northshore up to the shallow area and an eastward counter current along the Island (Fig. 5B). A layered circulation appeared to dominate in the deeper channel as inferred from the slope of the isopycnals with a westward flow above 80 m in the western part of the channel and an eastward flow at greater depth (Fig. 5B).

Cold water masses were observed along the Québec northern coast in May 1987 with a weak salinity discontinuity over the shallow area (Fig. 6A). Warmer and less saline water dominated the central portion of the northwest sector. A well defined frontal zone associated with the Gaspé current (interface between the St. Lawrence lower estuary and the Gulf) was apparent at the southern limit of the sector (Fig. 6A).

The isopycnal cross-sections in May 1987 indicated dense water near the surface, especially along the west to east transect where a less dense surface layer appeared to flow offshore with upwelling along the coast (Fig. 6B). In the north Anticosti sector a net north to south slope of the isopycnals was consistent with upwelling of colder water along the Québec north coast and subsequent strengthening of the eastward surface outflow along the north coast of the Island (Fig. 6B). The general hydrography of this sector was, however, relatively homogeneous and none of the features (e.g. circular-cells) observed in June 1986 were present.

Cluster Analysis

Geographical illustration of the multivariate clustering analysis shows a pattern consistent with the major features inferred from the *T-S* data (Fig. 7A, 7B). The water masses east of Beaugé Bank were well discriminated in June 1986 and the heterogeneity of the north Anticosti sector and the discrete water mass of the shallow area was revealed (Fig. 7A). The con-

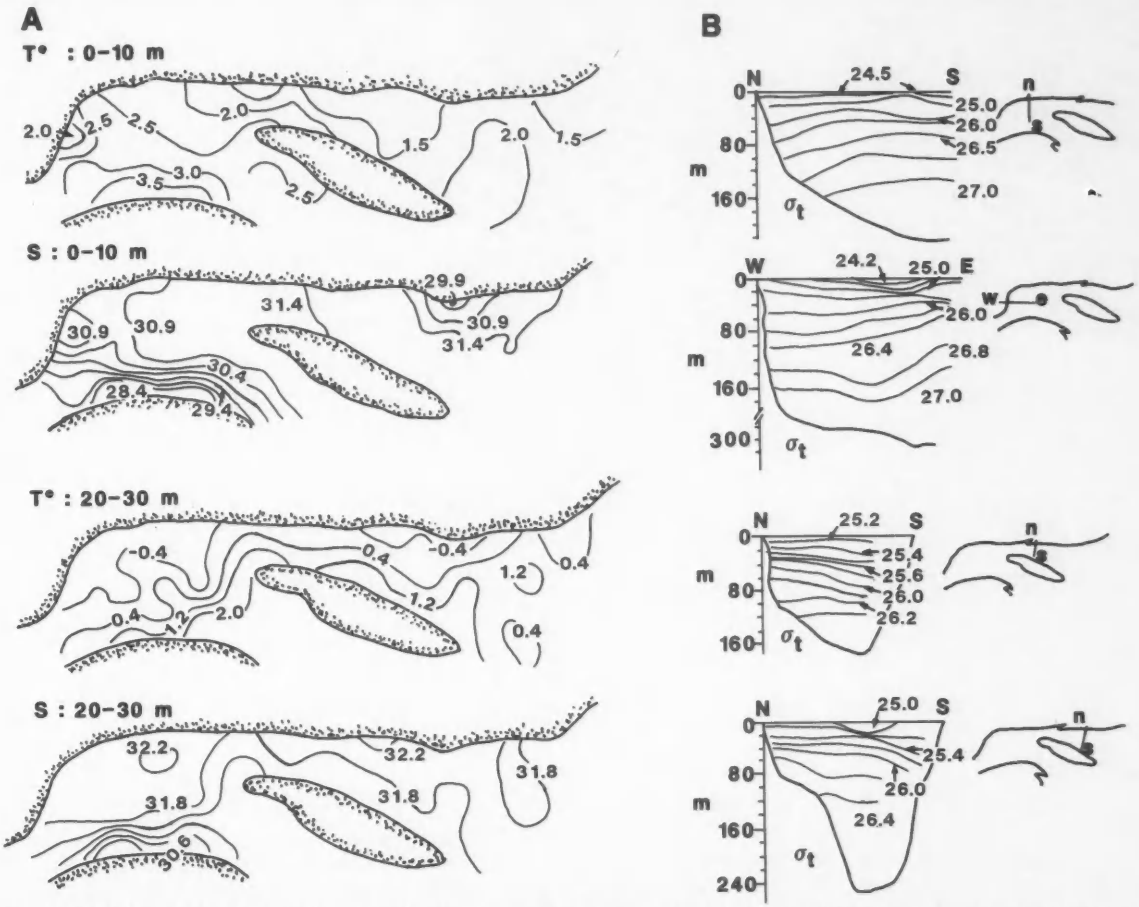

FIG. 6. (A) Horizontal distribution of the upper mixed-layer average temperature and salinity in May 1987 in the northwest and north Anticosti sectors of the northern Gulf of St. Lawrence. (B) Isopycnal distribution at cross-sections in the northwest and north Anticosti sectors.

cordance of patterns between the numerical description and the distribution of the *T-S* data infer that this method is robust in summarizing the hydrographic heterogeneity of the sectors. The same concordance between the analyses was observed for 1987 (Fig. 7B). These results served to distinguish the hydrographic structures used to test the relations between local hydrography and variability in larval abundance; i.e. Northwest I (coastal water mass in the northwest), Northwest II (central northwest sector and Gaspé current), N-Anticosti I (north of the Island), and N-Anticosti II (east end of the Island to Beaugé Bank) (Fig. 7A, 7B).

Larval Distribution and Hydrography of the Sectors

The Mantel test (Table 3) generally confirmed the results from the Moran's coefficient correlograms (Fig. 4). The biological variable (stage I larvae) was structured spatially in May 1986, but no correlation was found with the stage II larvae in June 1986 (Table 3). The significant relationships between the hydrographic description of the sampling areas and geographic location (N-Anticosti I; June 1986, and Northwest I; May 1987, Table 3) were consistent with the hydrographic heterogeneity. However, when the hydrographic heterogeneity was substituted for the geographical distances no correspondence was found with the biological variate except in areas of relatively high and homogeneous larval concentration (Northwest I and N-Anticosti II, May 1987, Table 3).

Discussion

Integrity of the Gulf Shrimp Groups and Stocks

Sinclair and Iles (1988) hypothesized that isolated spawning, emergence, and regional retention of the larvae (geographical life-history closure) should be the ultimate regulatory agents of population definition and control of abundance. In this context, general inferences can be made. First, the actual grouping of the fisheries assessment data infer the presence of different *P. borealis* stocks in each sector of the northern Gulf of St. Lawrence (Savard et al. 1988). Second, the high proportion of berried females shrimp in spring (April, May) in each sector suggest the presence of local reproductive units (Savard et al 1988). Third, the identification of discrete larval populations in the sectors would also suggest the recognition of the northern Gulf shrimp aggregations as independent biological

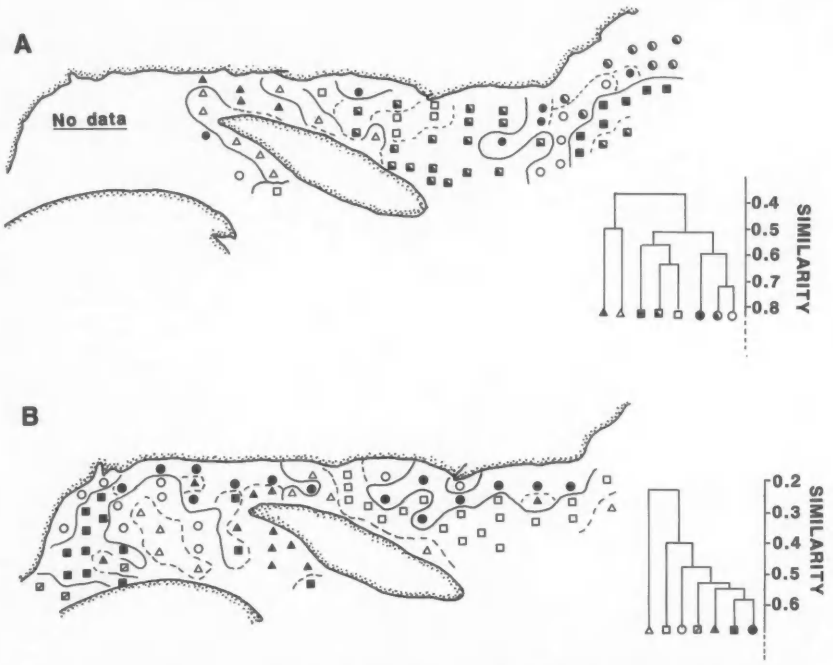

FIG. 7. Geographic representation of the cluster analyses performed on the hydrographic data: (A) north Anticosti and northeast sectors — June 1986; (B) northwest and north Anticosti sectors May 1987.

populations. It is the third inference on which we have concentrated in this study.

Localized larval emergence of *P. borealis* within each sector (and, at least in the northwest, coincident with the known spring distribution of female shrimp) was evident during the sampling periods of this study. However, following emergence, the first pelagic stages were widely distributed and exchanges among sectors could not be ruled out. Exchanges probably occurred in May 1987 from the northwest to north Anticosti sector, and possibly in June 1986 along the Québec northshore between the northeast and north Anticosti sector. This was apparent even though the hydrographic structure appeared to favour isolation of larval groups. The results of classification analysis of the hydrographic data are consistent with relative isolation of the sectors with transition zones between the northwest and Anticosti sectors over the shallow area, and between the northeast and Anticosti sectors along the Québec northshore. Therefore, given the possibility that mixing of larval groups may occur, the current management grouping of shrimp in the Gulf may not represent independent biological populations but geographical reproductive units of a single population in the northern Gulf of St. Lawrence.

The frequency and importance of larval transport through the shallows and of larval loss by advection outside the sectors cannot be determined from our data. In addition, the absence of correlation between the larval distribution pattern and the hydrography of the sectors prevents the description of larval dispersion from analysis of the water mass characteristics. The results expected from a hydrodynamical study of the Anticosti region (D. Lefaivre, unpubl. data) may provide further information to assess the importance of mixing and the general circulation in the area.

Nevertheless, certain interpretations are possible, based on the hydrographic patterns observed in the sectors. The west to east orientation (especially strong in May 1987) of the surface isotherms is indicative of east to west mass transport dominating over north to south transport. This is expected considering the configuration of the coasts in the region and the resulting stronger influence of westerly and easterly winds on water movement. Hence, the presence of high larval abundance over the shallow area in May 1987 and the displacement eastward of the north Anticosti larval group, may have resulted from incidental wind-driven westward advection of the surface water masses. However, the presence of cold water at the surface resulting from recently melted ice on the coast may have exaggerated the apparent thermal uniformity. The latter interpretation is supported by the salinity discontinuity observed over the shallow zone and by the separation of the sectors in the cluster analysis. Furthermore, no evidence of similar exchanges can be inferred from the May 1986 and June 1986 sampling periods. In fact, the hydrographic structure in the upper layer of the north Anticosti and northeast sectors in June 1986 provide possible mechanisms (front, circular current) for the maintenance of larval groups over Beaugé Bank and at the western end of the Anticosti sector.

Some evidence of two-layered deep circulation was apparent in the north Anticosti sector. It may be suggested that the estuarine like circulation is responsible for seaward drift of the early larval stages followed by a re-introduction of the older stages with the deep current, as suggested for larvae of decapod crustacean in some coastal areas (see references in Weinstein

TABLE 3. Standardized Mantel statistic 'Z' and significance probabilities '*P*' between geographic, hydrographic, and biological distances matrices, for each sector and sampling period. (* = $\{1-P\} < 0.05$ for positive *Z*)

Period	Sector	Model	*Z*	*P*
May 1986	Northwest	Bio-Geo	0.429	0.97*
	N-Anticosti	Bio-Geo	0.637	0.98*
		Hyd-Geo	0.665	0.97*
		Bio-Hyd	0.099	0.63
June 1986	Northwest	Bio-Geo	−0.400	0.12
	N-Anticosti I	Bio-Geo	0.256	0.39
		Hyd-Geo	0.946	0.99*
		Bio-Hyd	0.167	0.76
	N-Anticosti II	Bio-Geo	0.091	0.60
		Hyd-Geo	0.117	0.69
		Bio-Hyd	−0.556	0.10
	Northeast	Bio-Geo	−0.516	0.09
		Hyd-Geo	0.319	0.94
		Bio-Hyd	0.124	0.67
May 1987	Northwest I	Bio-Geo	−0.197	0.37
		Hyd-Geo	0.515	0.95*
		Bio-Hyd	0.656	0.98*
	Northwest II	Bio-Geo	−0.589	0.11
		Hyd-Geo	−0.156	0.43
		Bio-Hyd	0.159	0.76
	N-Anticosti I	Bio-Geo	0.343	0.86
		Hyd-Geo	0.360	0.89
		Bio-Hyd	−0.239	0.39
	N-Anticosti II	Bio-Geo	0.251	0.87
		Hyd-Geo	0.154	0.71
		Bio-Hyd	0.537	0.98*

1988). However, the depth distribution of the late larval stages (stage III+) was not investigated in our study and the results, from single-point measurements of the water characteristics, are insufficient to describe the average long-term circulation pattern in the northern region of the Gulf. In any case, the estimated residence time of the surface water masses (approximately 1 yr) in the northern Gulf (Stevens 1974; Bugden 1981), is well beyond the time scale of ~90 d required for complete larval development of shrimp.

Our analysis indicates that larval groups emerge and may be maintained at discrete locations within a sector (development from zoea I to zoea III implies a period of at least 25 d at the temperatures observed in the surface layer). We therefore suggest that the populations in each sector were locally produced and that mixing between sectors or advective loss were relatively unimportant, especially in 1986. The survey area is the only region of the Gulf where *P. borealis* are found in significant concentrations. It is unlikely that larval immigration from other regions contributed to the sampled populations or that nursery areas exist outside the region. Hence, it appears that larvae surviving within a sector are responsible for the annual level of recruitment to the local shrimp aggregation, in accord with the present management strategy. The distribution of young males (< 2 yr) in the shallow regions near the Québec north coast in the northwest and north Anticosti sectors (Simard and Savard 1990) is also evidence of local source recruitment.

Larval Distribution and Hydrographic Pattern

Studies dealing with the spatial distribution of decapod larvae generally describe a wide, physically controlled dispersion of the early stages. Parsons et al (1986) noted that *P. borealis* larvae were broadly distributed along the Labrador coast, presumably a result of transport by the Labrador current. The dispersal of *P. jordani* larvae offshore and along the Oregon coast appears to be controlled by the extent of upwelling (Rothlisberg and Miller 1983). Harding and Trites (1988) presented evidence that lobster on Browns Bank (southwest Nova Scotia) released their young such that the local circulation will broadly distribute the larvae throughout the Gulf of Maine. Finally, Hudon et al (1986) were able to explain the distribution of lobster larvae around Iles-de-la-Madeleine (Québec) from residual current patterns. In contrast, *P. borealis* larval distribution in Kachemak Bay (Alaska) was not entirely related to the water mass circulation in the area, and larval behaviour may be significant in determining distribution (Haynes 1983).

The Gulf of St. Lawrence is a semi-enclosed sea, and has relatively low hydrodynamic energetics (Pingree and Griffiths 1980): tides and tidal current are of low amplitude (<1 m and <20 $cm \cdot s^{-1}$, respectively). Winds play a dominant role in the physical dynamics of the surface layer (Tang 1980), but the formation of wind-induced circulation patterns are transient phenomena. Initially, larvae may emerge in a well-defined water mass. However, as larvae are not a conservative property of that water mass, subsequent identification of the larval group will depend on the correspondence of scale between the persistence of the water mass in the system (i.e. mixing time) and the dynamics of the larval survival.

Our analyses show that there was no clear relationship between hydrographic structures and the pattern of distributions of the larvae within the diverse sectors of the Gulf. The Mantel test we used has been shown to be a simple and reliable measure of correlation between spatially dependent variables for many ecological situations (Legendre and Troussellier 1988; Sokal et al. 1987; Mackas 1984).

Larval shrimp distribution during June 1986 also showed no geographic structure. The synoptic sequence of the pelagic developmental phases (May 1986 and June 1986) indicated that the abundance distribution evolved from a highly structured pattern to an apparently random one. This suggests that, in the northern Gulf, the processes of larval development and mortality may be more important than local hydrodynamics in determining the spatial structure of the distribution. Consequently, on a seasonal basis, the dispersion of larval shrimp groups cannot easily be inferred from analysis of the hydrographic structure of the sectors.

It may be argued that sampling the spatial distribution of any ecological variable is subject to spatial aliasing (resulting from the observation scale) and the resultant pattern reflects only the spatio-temporal bias of the sampling protocol. Moreover, shrimp larvae cannot be aged on a daily basis (the first intermoult period is 10–14 d at ~4°C, approximately the time required to sample the entire grid), and so it is not possible to identify precisely the emergence site of any group of larvae and their fine scale pattern of dispersal. Consequently, a broad distribution of release sites within sectors may mask any small-scale hydrographic control of the larval distribution. It remains, however, that the independent hydrographic and biological variables were recorded concurrently at each station and that no correlation was observed between the spatial patterns of variability in each variable. This suggests that the sources of heterogeneity are different; e.g. mortality rate and time-dependent mixing of the surface water masses. This interpretation does not contradict the fact that large scale hydrographic features

(front at Beaugé Bank and at the shallow zone) apparently determine the broad limits of larval distributions among sectors.

Our results suggest that future studies of recruitment variability among the Gulf of St. Lawrence shrimp groups should examine factors influencing the probability of survival of individual larvae (e.g. condition index) in specific water masses of the northern Gulf. This is in accord with Legendre and Demers (1984) and Legendre et al (1986) who suggested that the study of planktonic population dynamics should concentrate on physiological rates and biochemical condition as more robust means (relative to abundance estimates) of assessing individual and population response to the dynamics of the environment.

Acknowledgments

We thank Mr. François Gregoire for his generous help during the cruises and the supervision of the laboratory analysis. We also thank the captains and crew of the M. V. *Alfred Needler* and *Lady Hammond* for their help and patience during the cruises; and Dr. C. T. Taggart for his helpful comments and criticisms. Finally, the comments of Dr. C. Hudon and one anonymous referee were instrumental to the improvement of the manuscript.

References

BERKELEY, A. A. 1931. The post-embryonic development of the common Pandalids of British Columbia. Contrib. Can Biol. 6: 79–163.

BUGDEN, G. L. 1981. Salt and heat budgets for the Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 38: 1153–1167.

HARDING, G. C., AND R. W. TRITES. 1988. Dispersal of *Homarus americanus* larvae in the Gulf of Maine from Browns Bank. Can. J. Fish. Aquat. Sci. 45: 416–425.

HAYNES, E. 1979. Description of larvae of the Northern shrimp, *Pandalus borealis*, reared in situ in Kachemak Bay, Alaska. U.S. Fish. Bull. 77(1): 157–173.

1983. Distribution and abundance of larvae of King Crab, *Paralithodes camtschatica*, and Pandalid shrimp in the Kachemak Bay area, Alaska, 1972 and 1976. NOAA Tech. Rep. NMFS SSRF-765: 35 p.

HUDON, C., P. FRADETTE, AND P. LEGENDRE. 1986. La répartition horizontale et verticale des larves de homard (*Homarus americanus*) autour des Iles-de-la-Madeleine, golfe du Saint-Laurent. Can. J. Fish. Aquat. Sci. 43: 2164–2176.

LEGENDRE, P. 1987. Organisation spatiale des communautés biologiques: importance pour les théories de gestion des écosystèmes. *In* Comptes-rendus de la Conférence européenne "Diversité biologique — Un défi pour la science, l'économie et la société". Dublin, Irlande, 4–6 mars 1987. Programme FAST, Commission des Communautés Européennes.

LEGENDRE, L., AND S. DEMERS. 1984. Towards dynamics biological oceanography and limnology. Can. J. Fish. Aquat. Sci. 41: 2–19.

LEGENDRE, L., S. DEMERS, AND D. LEFAIVRE. 1986. Biological production at marine ergoclines,p. 1–16. *In* J. C. J. Nihoul [ed.] Marine interfaces ecohydrodynamics. Elsevier Oceanography Series 42, Amsterdam.

LEGENDRE, L., ET P. LEGENDRE. 1984. Écologie numérique, tome 2 - La structure des données écologiques (2nd ed.). Collection d'Écologie-13-. Masson, Paris et Presses de l'Université du Québec. 335 p.

LEGENDRE, P., AND M. TROUSSELLIER. 1988. Aquatic heterotrophic bacteria: modelling in the presence of spatial autocorrelation. Limnol. Oceanogr. 33(5): 1055–1067.

MACKAS, D. L. 1984. Spatial autocorrelation of plankton community composition in a continental shelf ecosystem. Limnol. Oceanogr. 29(3): 451–471.

ODEN, N. L. 1984. Assessing the significance of a spatial correlogram. Geogr. Anal. 16(1): 1–15.

PARSONS, D. G., G. R. LILLY, AND G. J. CHAPUT. 1986. Age and growth of Northern shrimp *Pandalus borealis* off northeastern Newfoundland and southern Labrador. Trans. Am. Fish. Soc., 115: 872–881.

PIKE, R.B., AND D. I. WILLIAMSON. 1964. The larvae of some species of Pandalidae (Decapoda). Crustaceana 6: 265–284.

PINGREE, R. D. AND D. K. GRIFFITHS. 1980. A numerical model of the M_2 tide in the Gulf of St. Lawrence. Oceanol. Acta 3(2): 221–225.

POSGAY, A., AND R. R. MARAK. 1980. The MARMAP bongo zooplankton samplers. J. Northwest Atl. Fish. Sci. 1: 91–99.

ROHLF, F. J. 1987. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Applied Biostatistics Inc., Exeter Publishing, North Country Road, New York, NY.

ROTHLISBERG, P. C., AND C. B. MILLER. 1983. Factors affecting the distribution, abundance, and survival of *Pandalus borealis* (Decapoda, Pandalidae) larvae off the Oregon coast. U.S. Fish. Bull. 81(3): 455–472.

SAVARD, L., Y. LAVERGNE, ET J. LAMBERT. 1988. Évaluation des stocks de crevette *Pandalus borealis* du Golfe du Saint-Laurent. CSCPCA, Doc. Rech. 88/60, 49 p.

SHUMWAY, S. E., H. C. PERKINS, D. F. SCHICK, AND A. P. STICKNEY. 1985. Synopsis of biological data on the pink shrimp, *Pandalus borealis* Krøyer, 1838. NOAA Tech. Rep. NMFS 30. U.S. Department of Commerce, Washington, D.C. 57 p.

SIMARD, Y., AND L. SAVARD. 1990. Variability, spatial patterns and scales of similarity in size-frequency distributions of the northern shrimp (*Pandalus borealis*) and its migrations in the Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 47(4): 794–804.

SINCLAIR, M., AND T. D. ILES. 1988. Population richness of marine fish species. Aquat. Living Resour. 1: 71–83.

SMITH, P. E., AND S. L. RICHARDSON. 1977. Standard techniques for pelagic fish egg and larva surveys. FAO Fish. Tech. Pap. 175. Rome. 100 p.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy — the principles and practice of numerical classification. W. F. Freeman and Co., San Francisco, CA. 573 p.

SOKAL, R. R., AND N. L. ODEN. 1978. Spatial autocorrelation in biology. 1. Methodology. Biol. J. Linn. Soc., 10(2): 199–228.

SOKAL, R. R., N. L. ODEN, AND J. S. F. BARKER. 1987. Spatial structure in *Drosophila buzzatii* populations: simple and directional spatial autocorrelation. Am. Nat. 129: 122–142.

SOKAL, R. R., AND J. D. THOMSON. 1987. Applications of spatial autocorrelation in ecology, p. 431–466. *In* L. Legendre and P. Legendre [ed.] Developments in numerical ecology. NATO ASI Series, vol. G14. Springer–Verlag. Berlin.

STEVENS, D. M. 1974. Primary and secondary production in the Gulf of St. Lawrence. Marine Science Centre. McGill University. Canadian Contribution to the International Biological Program (CCIBP). Rep. 26.

STICKNEY, A. P., AND H. C. PERKINS. 1981. Observations on the food of the larvae of the Northern shrimp, *Pandalus borealis* Krøyer (Decapoda:Caridae). Crustaceana 40(1): 36–49.

TANG, C. L. 1980. Mixing and circulation in the Northwestern Gulf of St. Lawrence: a study of a buoyancy-driven current system. J. Geophy. Res. 85/C5: 2787–2796.

WEINSTEIN, M. P. [ED] 1988. Larval fish and shellfish transport through inlets. Am. Fish. Sco., Symp. 3. 165 p.

Temporary Residence by Juvenile Salmon in a Restored Estuarine Wetland[1]

David K. Shreffler, Charles A. Simenstad, and Ronald M. Thom

Wetland Ecosystem Team, Fisheries Research Institute, WH-10, University of Washington, Seattle, Washington 98195 USA

Shreffler, D. K., C. A. Simenstad, and R. M. Thom. 1990. Temporary residence by juvenile salmon in a restored estuarine wetland. Can. J. Fish. Aquat. Sci. 47: 2079–2084.

Juvenile Pacific salmon utilizing the recently restored Lincoln Avenue wetland system in the Puyallup River estuary, Tacoma, Washington, were studied during their spring seaward migration in 1987 and 1988. Mark/recapture experiments indicated that 0.06% of the outmigrating juvenile chum salmon (*Oncorhynchus keta*) and 0.59% of the outmigrating juvenile chinook salmon (*O. tshawytscha*) entered the wetland. Estimated residence times of individual juvenile chum salmon averaged approximately 2 d (range 1–9 d) and juvenile chinook salmon approximately 5 d (volitional) and 38 d (spray-marked) (total range 1–43 d). The restored wetland system currently provides habitat for the temporary residence of migrating juvenile chum and fall chinook salmon, but rigorous evaluation of the benefit of residency is constrained by the lack of data from comparable natural wetlands.

En 1987 et 1988, on a étudié les juvéniles de saumons du Pacifique qui fréquentent les marécages récemment restaurés de Lincoln Avenue dans l'estuaire de la Puyallup à Tacoma, dans l'État de Washington, lors de leur migration printanière vers la mer. Les expériences sur les poissons marqués et recapturés révèlent que 0,06% des juveniles de saumons keta (*Oncorhynchus keta*) se dirigeant vers la mer et 0,59% des juvéniles de saumon chinook (*Oncorhynchus tshawytscha*) se dirigeant vers la mer passent par le marécage. On estime que chaque juvénile de saumon keta y a passé en moyenne deux jours (pour des extrêmes de 1 à 9 jours) alors que le temps de séjour moyen des juvéniles de saumon chinook est d'environ cinq jours (spontanément) ou 38 jours (saumon marqué) (pour des extrêmes de 1 à 43 jours). Le système marécageux restauré offre actuellement un habitat temporaire pour les juvéniles de saumon keta et de saumon chinook qui entament leur migration mais le manque de données sur des zones marécageuses naturelles comparables fait qu'il est difficile d'évaluer de façon rigoureuse les avantages de ce séjour.

Received November 7, 1989
Accepted May 15, 1990
(JA354)

Reçu le 7 novembre 1989
Accepté le 15 mai 1990

Most anadromous Pacific salmon utilize estuarine wetlands of the Pacific Northwest as juveniles when migrating to the ocean, and as adults returning to spawn in freshwater. Understanding utilization of estuaries by juvenile Pacific salmon, especially in vanishing wetland habitats, has ecological and practical implications for salmon resource management and habitat protection. The extensive historical losses and alterations of wetland habitats in this region have undoubtedly diminished the function by many estuarine environments as temporary residence areas that once contributed to increased survival of juvenile salmon.

During the last century 98.6% of the native wetland habitat once present in the Puyallup River estuary has been destroyed through dredging, diking, and filling (Bortleson et al. 1980). The Lincoln Avenue wetland, which was restored in February 1986, provides the only emergent marsh habitat now available to migrating juvenile salmon in the Puyallup River estuary. The wetland was an excellent experimental habitat for assessing juvenile salmon utilization of restored wetlands because the narrow opening at the mouth enabled us to monitor the immigration and emigration of juvenile salmon. Our research focused on juvenile chum and fall chinook salmon because, of the five species of Pacific salmon, these two are most dependent upon estuaries (Dorcey et al. 1978; Healey 1982). This study had two objectives: (1) to experimentally determine what proportion of the juvenile chum and fall chinook salmon migrating down the Puyallup River entered the restored Lincoln Avenue wetland and (2) to estimate juvenile chum and fall chinock salmon residence times in the wetland.

[1]Contribution No. 821, School of Fisheries, University of Washington, Seattle, WA 98195, USA.

Study Area

In 1985–86, the 3.9-ha Lincoln Avenue wetland was constructed in the tidally influenced portion of the Puyallup River (122° 22′W, 47° 15′N) to replace a 3.9-ha wetland 1.6 km downstream which was filled for development (Fig. 1). The river dike was breached on February 20, 1986, following excavation of approximately 55 000 m^3 of solid waste landfill, construction of a containment dike, and contouring of the new wetland (Thom et al. 1987). In accordance with criteria established in negotiation with fish and wildlife resource agencies, 50% of the habitat area was designed to support juvenile salmon, 20% waterfowl, 10% shorebirds, 10% raptors, and 10% small mammals.

As defined by the Cowardin et al. (1979) classification sysem, the Lincoln Avenue wetland is a "persistent, emergent wetland within an intertidal estuarine system." In its present state of development, the restored wetland is a regularly flooded, brackish system comprised of tidal channels, mudflats, a transplanted sedge (*Carex lyngbyei*) marsh, a cattail

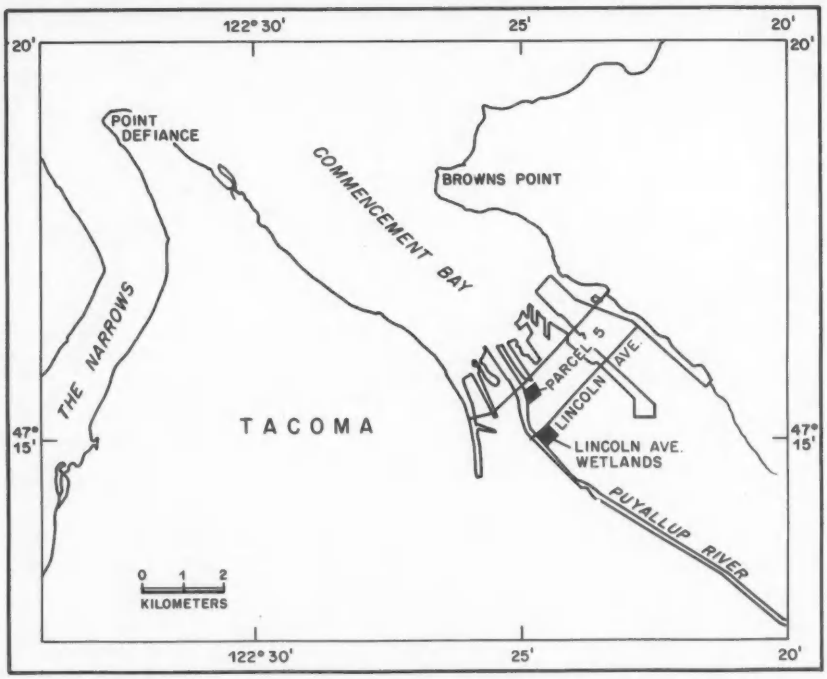

FIG. 1. Location of the Lincoln Avenue wetland and the Parcel 5 wetland which was filled for development, Puyallup River estuary, Tacoma, Washington.

(*Typha* spp.) marsh, a shrub-scrub swamp, riparian hardwoods, and an upland grassland. The tidal channels, mudflats, and central basin, which cover approximately 2.2 ha, provide the principal fish habitat. Surface salinity ranges from 0.14 to 7.2‰ in the wetland. The wetland system is unique in size, location, and design, and is currently the largest estuarine mitigation project in the state of Washington (Cooper 1987).

The Puyallup River is populated by six species of Pacific salmon, including fall and spring chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), pink (*O. gorbuscha*), chum (*O. keta*), cutthroat (*O. clarki*), and steelhead (*O. mykiss*). The Puyallup River has been estimated to produce over 270 000 adult salmon per year with an average spawning population of 63 000 fish (Washington Department of Ecology 1980). Although some small wild stocks do persist, the dominant salmonids in the system are hatchery-reared fall chinook and chum salmon from the Puyallup Indian Tribe hatchery at Clark's Creek and the Washington Department of Fisheries hatchery at Voight Creek (Table 1).

Materials and Methods

Beach seine sampling in 1986 indicated that juvenile salmon were entering the wetland within 2 mo of the breaching of the wetland dike (Thom et al. 1987). In 1987 and 1988, we systematically sampled the wetland fish assemblage and conducted experiments to determine juvenile chum and fall chinook salmon entrance into, and residence times within, the restored wetland.

General Sampling Design

To estimate the proportion of migrants entering the wetland, hatchery-reared chum salmon (1987) and fall chinook salmon (1988) were spray marked and released into Clark's Creek, a tributary of the Puyallup River 4.8 km upstream from the wetland. The spray-marked fish were recaptured alive at the mouth of the wetland in the inlet net of an inlet outlet-fyke net system constructed to quantify immigration to (fish captured in the inlet-fyke net) and emigration from (fish captured in the outlet-fyke net) the wetland. The inlet and outlet-fyke nets were checked daily at low slack tide from May 6 to June 16, 1987 and March 11 to June 30, 1988. Juvenile salmon in the inlet-fyke net were transferred to buckets and anesthetized with MS-222. Spray-marked fish were detected using a battery-operated ultraviolet light. To determine what these spray-marked fish had eaten in route from Clark's Creek to the wetland, they were preserved immediately in 10% buffered formalin for later gut content analysis (Shreffler et al. 1990). Unmarked fish were marked by clipping some combination of the ventral, caudal, or anal fins, and then released into the wetland.

Independent estimates of juvenile salmon wetland residence times were derived from: (1) fish that volitionally migrated downstream, were captured in the inlet-fyke net, were finclipped, passed over the inlet fyke net into the wetland, and were recaptured in the outlet-fyke net as they left the wetland; and (2) spray-marked fish, that were transported from the Puyallup Indian Tribe's hatchery, released directly into one of the tidal channels of the wetland, and recaptured in the outlet fyke net.

Using a 9.2-m floating beach seine with a 6-mm mesh bag and a solid core lead line, we also collected samples in the wetland's tidal channels on April 21, May 4, and May 5, 1988. Beach seine sampling was minimized because it resulted in high fish mortalities. After beach seine sampling on May 4, the fyke net sysem was removed from the mouth of the wetland. Four

TABLE 1. Summary of 1987–88 estimates of hatchery and wild juvenile chum and chinook salmon outmigrants for the Puyallup River, and recaptures of chum and chinook outmigrants in the inlet-fyke, outlet-fyke, and beach seines at the Lincoln Avenue wetland (LAW).

	Outmigrant estimates		Recaptures at the LAW					
			Inlet-fyke		Outlet-fyke		Beach seines	
	Hatchery[a]	Wild[b]	Marked	Unmarked	Marked	Unmarked	Marked	Unmarked
Chum								
1987	9 000[c]	6,000	5	0	91	49	0	44
1988	349 920	6,000	0	221	0	74	0	85
Chinook								
1987	1 556 995	9,000	0	14	0	2441	0	696
1988	1 719 000[d]	9,000	105	1936	51	281	18	430

[a]Puyallup Tribal Hatchery releases plus Washington Department of Fisheries releases.
[b]Based on mean total run sizes for 6 yr (1978–83).
[c]All spray marked and experimentally released (this study).
[d]17 800 were spray marked and experimentally released (this study).

of the six tidal channels were sampled by beach seine on May 5 to determine how many fish remained in the wetland despite having free access to the river (i.e. did the fyke net system artificially increase juvenile salmon residence times by preventing volitional emigration?).

Spray Marking

On May 2, 1987, approximately 10 000 juvenile chum salmon were spray marked (6,000 red, 4,000 yellow) at the Puyallup Indian Tribe's hatchery with a biologically inert, polystyrene, fine-grit, fluorescent pigment using the method of Bax (1983). Groups of approximately 300 chum salmon at a time were placed in a plexiglas marking trough, immediately sprayed with pigment for 8 s using a sandblasting gun connected to a compressed air tank regulated at 125 psi, and then carefully rinsed with a hose into separate net pens. Each experimental group was suspended in a net pen in a concrete raceway. Mortalities were counted the following day and one group of fish was held at the hatchery and checked for mark retention on May 3, June 1, and June 9, 1987.

On March 4, 1988, approximately 20 000 fall chinook salmon (10 000 red, 10 000 yellow) were spray marked using the same procedure as in 1987, except that the marked fish were held in three separate floating net pens in an earthen pond. The following day, mortalities were removed from the pens and a group of fish was held at the hatchery and checked for mark retention on March 6, 11, 20, and April 29, 1988.

Entrance

Approximately 9,000 spray-marked chum salmon ($\bar{x}$ = 44.4 ± 2.9 mm fork length [FL]; $\bar{x}$ = 0.65 ± 0.14 g wet wt.; yellow and red marks combined) were released at 1700 on May 7, 1987 into Clark's Creek and allowed to migrate downstream volitionally.

On March 14, 1988, approximately 17 800 spray-marked fall chinook salmon ($\bar{x}$ = 45.2 ± 3.2 mm FL; $\bar{x}$ = 0.90 ± 0.21 g wet wt.; yellow and red marks combined) were released at 1500 into Clark's Creek at the site where chum salmon had been released in 1987.

Residence Times

On May 21, 1987, 438 yellow-marked chum salmon ($\bar{x}$ = 52.4 ± 4.2 mm FL; $\bar{x}$ = 1.28 ± 0.30 g wet wt.) were released at approximately 1530 into channel 3 of the wetland on an incoming tide. A second group of 447 red-marked chum salmon ($\bar{x}$ = 53.7 ± 3.6 mm FL; $\bar{x}$ = 1.31 ± 0.30 g wet wt.) was released at 1700 into one of the tidal channels on May 27, 1987 just prior to high tide.

On March 23, 1988, approximately 1,000 red-marked fall chinook salmon ($\bar{x}$ = 48.2 ± 4.0 mm FL; $\bar{x}$ = 1.20 ± 3.88 g wet wt.) were released at 1600 into one of the tidal channels during an incoming tide.

Results

Spray Marking Mortality and Mark Retention

Spray marking mortalities were low: 5.1% in 1987 and 0.05% in 1988, relative to the group of control fish in 1988, which were netted and handled in exactly the same way but were not sprayed (0.20% mortality). Monitoring of mark retention in 1987 and 1988 indicated that pigment on some of the marked fish released for residence time experiments may have been difficult to detect, and that the pigment may not have been sufficient to last the entire period of the juvenile chum salmon (1987) and fall chinook salmon (1988) migrations (Fig. 2). In both years, less than 50% of the fish could be identified as marked after 30 d. Mark retention was better in 1987 than in 1988, but mortalities resulting from spray marking were higher in 1987.

Entrance

Of the 9,000 marked chum salmon released in 1987, five (0.06%) were recaptured in the inlet fyke net. In 1988, 17 800 marked fall chinook salmon were released and 105 (0.59%) were recaptured in the inlet-fyke net.

Residence Times

Residence times of recaptured chum salmon averaged 1.7 d (range 1–9 d) for the yellow-marked release group, and 2.6 d (range 1–5 d) for the red-marked release group (Table 2). Residence times of recaptured chinook salmon averaged 5.1 d (range 1–40 d) for fish that migrated downstream and entered the wetland volitionally. Residence times of the red-marked group released directly into the wetland, however, averaged 38.3 d (range 29–43 d) (Table 3).

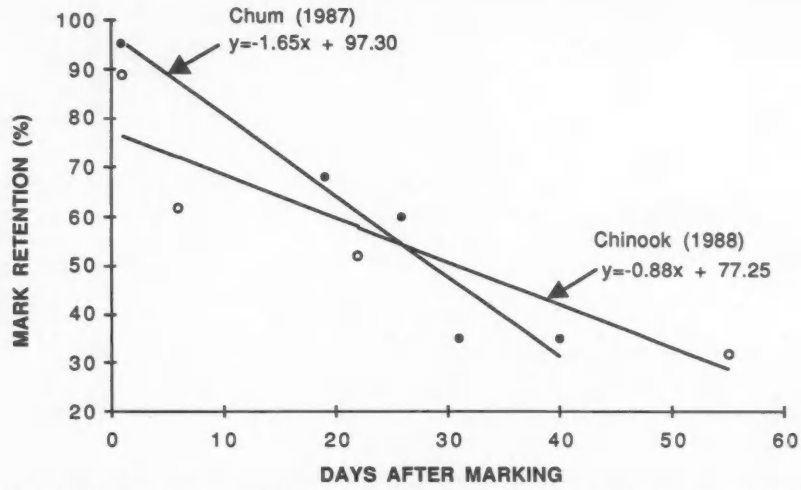

FIG. 2. Mark retention of juvenile chum salmon (1987; shaded circle) and fall chinook salmon (1988; clear circle) which were marked with a fluorescent pigment.

TABLE 2. Summary of 1987 spray-marked juvenile chum salmon releases, recaptures in the outlet-fyke, and individual residence times.

Date	Releases[a]	Recaptures	Residence times
May 21	438 yellow	—	—
May 22	—	39 yellow	1 d
May 23	—	31 yellow	2 d
May 24	—	—	—
May 25	—	—	—
May 26	—	—	—
May 27	447 red	—	—
May 28	—	8 red	1 d
May 29	—	2 yellow	8 d
	—	1 red	2 d
May 30	—	1 yellow	9 d
	—	8 red	4 d
May 31	—	1 red	5 d
June 1–10	—	—	—
	Total	73/438 yellow	$\bar{x}$ = 1.73 ± 1.45
		18/447 red	$\bar{x}$ = 2.61 ± 1.53
		91/995	$\bar{x}$ = 1.90 ± 1.51

[a]Released into tidal channel 3 of the wetland.

As of April 20, 1988, none of the red-marked fall chinook released 29 d earlier had been recaptured. On April 21, the two deepest tidal channels were sampled by beach seine, and six of the original fall chinook red-marked release group were recaptured along with 106 additional unmarked chinook. A total of 94 more unmarked fall chinook were seined from channel 4 on May 4.

The fyke net system was removed from the mouth of the wetland after beach seine sampling on May 4. On May 5, four of the six tidal channels were sampled by beach seine, and 12 red-marked fall chinook salmon and 230 unmarked chinook were collected and preserved, indicating that many juvenile salmon remained in the wetland even when given free access to the river. Mean residence times of the fall chinook red release group may have been even longer if it had not been necessary to preserve a significant percentage of the fish because of beach seining mortalities.

TABLE 3. Summary of 1988 fin-clipped and spray-marked juvenile fall chinook salmon releases, recaptures, and individual residence times. With the exception of the 1,000 red spray marks released directly into the wetland on March 23, all of these "releases" were outmigrants captured in the inlet-fyke net, fin-clipped, and released into the wetland; fin clip abbreviations: lv = left ventral, rv = right ventral, ad = adipose, lc = lower caudal, uc = upper caudal, an = anal; (bs) = beach seine recaptures; all other fish were recaptured in the outlet-fyke net; fish with residence times <1 d were released and recaptured the same day. Mean residence time (volitional): 5.06 ± 8.09 d, (red release group): 38.33 ± 6.60 d.

Date	Releases	Recaptures	Residence times
March 12	12 lv	—	—
March 13	17 lv	—	—
March 14	18 rv	—	—
March 15	38 nc	—	—
March 16	18 ad	—	—
March 17	207 lc	10 lc	<1 d
		14 lv	5–6 d
		4 rv	3 d
		2 ad	1 d
March 18	36 uc	10 lc	1 d
		3 uc	6–7 d
March 19-20	—	—	—
March 21	—	1 lc	4 d
March 22	—	—	—
March 23	1,000 red	2 lc	6 d
March 24–29	—	—	—
March 30	37 an	—	—
March 31–April 3	—	—	—
April 4	—	2 an	5 d
April 5–20	—	—	—
April 21	—	1 uc (bs)	34 d
		1 lc (bs)	35 d
		1 lv (bs)	40 d
		6 red (bs)	29 d
April 25–29	—	—	—
April 30–May 3	—	—	—
May 4	—	—	—
May 5	—	12 red (bs)	43 d

TABLE 4. Individual residence times (days) of juvenile chum and fall chinook salmon in Pacific Northwest estuarine wetlands.

Estuary	Chum	Chinook	Source
Sixes River (OR)		~93	Reimers 1973
Lymn Creek (BC)	7–14		Mason 1974
Nanaimo River (BC)	0–9		Healey 1979
Nanaimo River (BC)		~25	Healey 1980
Skagit River (BC)	2–4	3–6	Congleton et al. 1981
Fraser River (BC)	1–11	7–62	Levy and Northcote 1982
Campbell River (BC)		40–60	Levings et al. 1986
Puyallup River (WA)	1–9	1–43	Shreffler et al. 1990

The mark retention vs. time regression analysis indicated that less than 50% of the marked chinook held at the hatchery had detectable marks by April 21, 1988, and less than 32% by May 4 (Fig. 2). Thus, many "unmarked" chinook salmon (106 collected on April 21, 94 on May 4; and 230 on May 5) probably had undetectable marks.

Discussion

Chum and fall chinook salmon utilization of the Lincoln Avenue wetland for temporary residence was of particular interest because 50% of the restored wetland area was intended to support juvenile salmon. Estimated individual residence times in the restored wetland ranged from 1–9 d for chum and 1–43 d for fall chinook.

Chum salmon typically migrate downstream immediately after emergence, primarily at night, close to shore, and near the surface (Simenstad et al. 1982). Although estuarine rearing of chum salmon occurs in some systems (Healey 1979, 1982), extended rearing in brackish water is uncommon (Simenstad et al. 1982). The brief mean residence time estimated for chum salmon (<2 d) in 1987 suggests that residence in brackish habitats may be naturally abbreviated in the Puyallup River estuary. In addition, the rapid seawater acclimation of chum fry relative to chinook fry (Weisbart 1968; Folmar and Dickhoff 1980) may explain the shorter mean residence times of juvenile chum salmon within the brackish wetland. Iwata and Komatsu (1984) found that acclimation to isotonic estuary water for 12 h was sufficient for efficient adaptation of chum salmon to seawater, whereas Clarke and Shelbourn (1985) reported that 3–4 mo were necessary for fall chinook salmon adapation to seawater.

Like chum salmon, chinook salmon typically exhibit nearshore, nocturnal downstream migrations, but the age, size, and timing of downstream juvenile chinook migrants are highly variable for the different races (fall, winter, and spring). The extended residence times of juvenile fall chinook (up to 43 d) found in this study are among the longest described for any wetland and the first in a restored wetland (Table 4). The mean length of 12 recaptured chinook, which resided in the wetland for 43 d, increased 15.9 mm (0.37 $mm \cdot d^{-1}$), and their mean weight increased 2.24 g (5.2 $mg \cdot d^{-1}$). In the Nanaimo River estuary, fall chinook salmon grew about 1.3 $mm \cdot d^{-1}$ and 5.7 $mg \cdot d^{-1}$ (Healey 1980). The shorter residence time estimates for fall chinook that entered the wetland volitionally ($\bar{x}$ = 5.06 ± 8.09 d) relative to the fish released in the wetland ($\bar{x}$ = 38.33 ± 6.60 d) may have resulted from the following: (1) the fish that entered the wetland from the river may have already begun their physiological adaptation to saltwater; (2) the fish released in the wetland were never given the opportunity to forage in the river and hence had no way of assessing the foraging value of the river relative to the wetland; (3) the two groups may have been affected differently by river discharge or tidal effects; and (4) the behavior of the two groups may have been altered by the fyke-nets (e.g. net avoidance).

Although juvenile salmon foraged and grew while temporarily residing in the Lincoln Avenue wetland (D. K. Shreffler, C. A. Simenstad, and R. M. Thom, in preparation), interpretation of the system's status as a nursery area remains tenuous because meager comparable data are available from other wetlands in the region, and because the wetland is probably still undergoing a colonization process typical of perturbed or nascent habitats.

Acknowledgments

This study was funded by the Port of Tacoma. Special thanks go to Joe Miyamoto, Tom Demming, and Herman Dillan of the Puyallup Indian Tribe, who graciously provided juvenile chum and chinook salmon for experiments and logistical assistance throughout the project. Cliff Whitmus taught us the art of spray marking juvenile salmon. Tim Dalton, José Obra, Jim Bolger, John Stadler, Alan Olson, Mary Ruckelshaus, Richard Davis, Christine Andrews, Tom Quinn, Theresa Armetta, Mike Grossmann, Fran Yeatts, Debbie Olden, Jay Orr, John Shields, and Cyrus Zabetian were invaluable field assistants. Bruce Snyder helped locate necessary sampling gear at Big Beef Creek Research Station. Chuck Baransky and Pam Abrahamson provided summaries of Washington Department of Fisheries hatchery releases of juvenile salmon into the Puyallup River and its tributaries. Marcus Duke, through his editorial comments and suggestions, made this a far more readable manuscript.

References

BAX, N. J. 1983. Early marine mortality of marked juvenile chum salmon (*Oncorhynchus keta*) released into Hood Canal, Puget Sound, Washington, in 1980. Can. J. Fish. Aquat. Sci. 40: 426–435.

BORTLESON, G. C., M. J. CHRZASTOWSKI, AND A. K. HELGERSON. 1980. Historical changes of shoreline and wetlands at eleven major deltas in the Puget Sound region, Washington. Hydrological Investigations Atlas HA-617. U.S. Geological Survey, Denver, CO. 12 sheets.

CLARKE, W. C., AND J. E. SHELBOURN. 1985. Growth and development of seawater adaptability by juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) in relation to temperature. Aquaculture 45: 21–31.

CONGLETON, J. L., S. K. DAVIS, AND S. R. FOLEY. 1981. Distribution, abundance and outmigration timing of chum and chinook salmon fry in the Skagit salt marsh, p. 153–163. *In* E. L. Brannon and E. O. Salo [ed.] Salmon and trout migratory behavior symposium. School of Fisheries, Univ. Washington, Seattle, WA. 309 p.

COOPER, J. W. 1987. An overview of estuarine habitat mitigation projects in Washington State. Northwest Environ. J. 3: 112–127.

COWARDIN, L. M., V. CARTER, F. C. GOLET, AND E. T. LAROE. 1979. Classification of wetlands and deepwater habitats of the United States. U.S. Fish Wild. Serv. Biol. Serv. Program FWS/OBS-79/31: 103 p.

DORCEY, A. H. J., T. G. NORTHCOTE, AND D. V. WARD. 1978. Are the Fraser marshes essential to salmon? Univ. B.C. Westwater Res. Cent. Tech. Rep. 1: 29 p.

FOLMAR, L. C., AND W. W. DICKHOFF. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21: 1–37.

HEALEY, M. C. 1979. Detritus and juvenile salmon production in the Nanaimo estuary: I. Production and feeding rates of juvenile chum salmon (*Oncorhynchus keta*). J. Fish. Res. Board Can. 36: 488–496.

1980. Utilization of the Nanaimo River estuary by juvenile chinook salmon, *Oncorhynchus tshawytscha*. Fish. Bull. 77: 653–668.

1982. Juvenile Pacific salmon in estuaries: the life support system, p. 315–341. *In* V. S. Kennedy [ed.] Estuarine Comparisons. Academic Press, New York, NY.

IWATA, M., AND S. KOMATSU. 1984. Importance of estuarine residence for adaptation of chum salmon (*Oncorhynchus keta*) fry to seawater. Can. J. Fish. Aquat. Sci. 41: 744–749.

LEVINGS, C. D., C. D. MCALLISTER, AND B. D. CHENG. 1986. Differential use of the Campbell River estuary, British Columbia, by wild and hatchery-reared juvenile chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 43: 1386–1397.

LEVY, D. A., AND T. G. NORTHCOTE. 1982. Juvenile salmon residency in a marsh area of the Fraser River estuary. Can. J. Fish. Aquat. Sci. 39: 270–276.

MASON, J. C. 1974. Behavioral ecology of chum salmon (*Oncorhynchus keta*) in a small estuary. J. Fish. Res. Board Can. 31: 83–92.

REIMERS, P. E. 1973. The length of residence of juvenile fall chinook salmon in the Sixes River, Oregon. Oreg. Fish Comm. Res. 4: 1–43.

SIMENSTAD, C. A., K. L. FRESH, AND E. O. SALO. 1982. The role of Puget Sound and Washington coastal estuaries in the life history of Pacific salmon: an unappreciated function, p. 343–364. *In* V. S. Kennedy [ed.] Estuarine Comparisons. Academic Press, New York, NY.

THOM, R. M., C. A. SIMENSTAD, AND E. O. SALO. 1987. The Lincoln Street wetland system in the Puyallup River estuary, Washington. Phase I Report: construction and initial monitoring, July 1985–December 1986. Fish. Res. Inst., Univ. Washington, Seattle, WA. FRI-UW-8706: 85 p.

WASHINGTON DEPARTMENT OF ECOLOGY. (WDOE). 1980. Puyallup River basin instream resource protection program including administrative rules. West. Washington Instream Resour. Prot. Prog. 6: 42 p.

WEISBART, M. 1968. Osmotic and ionic regulation in embryos, alevins, and fry of the five species of Pacific salmon. Can. J. Zool. 46: 385–397.

Measuring Fleet Capacity and Capacity Utilization

Courtland L. Smith

Department of Anthropology, Oregon State University, Waldo 238, Corvallis, Oregon 97331-6403, USA

and Susan S. Hanna

Department of Agricultural and Resource Economics, Oregon State University, Ballard Extension Hall 213, Corvallis, Oregon 97331-3601, USA

Smith, C. L., and S. S. Hanna. 1990. Measuring fleet capacity and capacity utilization. Can. J. Fish. Aquat. Sci. 47: 2085–2091.

Fishing capacity measures the capability to catch fish. Capacity utilization is the actual effectiveness in catching fish; the ratio of catch to capacity. The factors comprising a fleet's capacity are the number and size of vessels, the technical efficiency of those vessels, and the time commitment of fishermen. Knowing the capacity utilization of a fleet is critical for evaluating management alternatives. This is true regardless of whether fisheries are managed with fleet controls or with individual controls. We illustrate the measurement of capacity utilization with the Oregon bottom trawl fishery, in which fleet capacity quadrupled between 1976 and 1982. From 1982 to 1985 capacity declined 30% due to economic problems in the industry and vessels finding better opportunities elsewhere. Capacity measurement is necessary to assess management-induced changes in controlled fisheries. Controlling capacity requires addressing all the elements associated with capacity growth. Examination of the components of fleet capacity reveals an array of management options.

La capacité de pêche mesure la faculté de capturer du poisson. Le coefficient d'utilisation de la capacité traduit l'efficacité de l'effort de pêche; il correspond au rapport entre les captures et la capacité de pêche. Les facteurs qui influencent la capacité d'une flottille sont le nombre et le tonnage des navires, leur efficacité technique ainsi que le temps que les pêcheurs consacrent à l'effort de pêche. Il est essentiel de connaître le coefficient d'utilisation de la capacité d'une flottille pour pouvoir en évaluer les possibilités de gestion. Cela demeure vrai que les pêcheries soient gérées en contrôlant les flottilles ou des unités. Nous illustrons la mesure du coefficient d'utilisation de la capacité par l'exemple de la pêche au chalut de fond pratiquée au large de l'Orégon, pour laquelle la capacité de la flotte a quadruplé entre 1976 et 1982. Entre 1982 et 1985, cette capacité a diminué de 30% à la suite des problèmes économiques qu'a connus l'industrie et parce que les navires ont trouvé de meuilleures zones de pêche ailleurs. Il est nécessaire de mesurer la capacité pour pouvoir évaluer les changements qui résultent de la gestion dans les pêcheries réglementées. La réglementation de la capacité doit porter sur tous les éléments en rapport avec la croissance de cette capacité. L'étude des composantes de la capacité d'une flotte révèle toute une gamme d'options de gestion.

Received July 24, 1989
Accepted June 1, 1990
(JA245)

Reçu le 24 juillet 1989
Accepté le 1 juin 1990

The general pattern in most fisheries is for harvest capacity to outstrip the fish stock's reproductive capacity. Historically, fishery managers have used input controls such as seasons, gear restrictions, closed areas, and license limitations to constrain the use of fishing capacity. Economic analysis shows that open-access fisheries, even when managed to take only the allowable catch, have more capacity than is necessary. Effort controls have not been successful in controlling fishing capacity, or in protecting fish stocks from excessive harvest. To correct problems of over harvest and over capacity, some fishery management systems are beginning to shift to individual quota systems. The idea of an individual quota is to control output and let market factors adjust fishing capacity by removing incentives for "capital stuffing"and the "race for fish."

A basic assumption using individual quotas to control catch is that the market will alter fishing capacity by selecting the most efficient fishing fleet. The expectation is that capacity utilization, the ratio of catch to capacity, should improve under an individual quota system. To track fleet changes, managers need a method for assessing fishing capacity. Since individual quota systems have only recently become a part of fishery management, we illustrate our conceptualization of capacity in a fishery which has been evolving under open access, the Oregon bottom trawl fishery. In the Oregon bottom trawl fleet, the pattern of capacity growth and decline has been particularly volatile.

Components of Fishing Capacity

Conversational expressions (Duranti 1985; Mulkay 1985) suggest capacity has four components: one is the number of fishing vessels, second is the size of each vessel, third is the technical efficiency of vessel operation, and the time spent fishing is the fourth.

In a fishery, if the number of fishing vessels increases, the "number" component of capacity increases. The statement,"too many fishermen catching too few fish," reflects the problem of excess numbers in a fishery.

The size of vessels comprises the second component of capacity. A typical pattern in the development stage of open access commercial fisheries has been for new fishing vessels to be larger than those already fishing: "You have to get big

or get out." Fishing vessels get larger in order to catch more fish, to catch fish before their competitors, and to fish stocks and grounds not previously accessible.

We separate number and size to distinguish two mechanisms by which fishing fleets grow. For example, the salmon limited entry program in British Columbia controlled the number of salmon vessels but not their size (Campbell 1972; Pearse and Wilen 1979). The result was greatly increased capacity due to small vessels being replaced by much larger ones. A similar dynamic of replacing existing vessels with more efficient and capital-intensive vessels has been repeated in other vessel limited entry programs and has resulted in increases in both fleet capacity and effective fishing effort (Keen 1973; Adasiak 1978; Meany 1978).

Capacity also increases when fishermen use equipment more effectively. "Boats don't fish, people do." This component we call the technical efficiency aspect of capacity. Fishermen improve their fishing gear, add electronic devices, learn more about environmental conditions, and invent new fishing techniques to make their vessels capable of catching more fish at a faster rate.

A temporal dimension is the fourth component of fleet capacity: "Time is money." Using a vessel, rather than a net, trap, or other item of fishing gear, means the time unit must account for the number of opportunities fishermen have to catch fish. Catch data are recorded for each trip. To calculate an annual fleet capacity, the maximum number of trips the average vessel makes per year must be estimated.

The elements of capacity derived from fishermen's conversational expressions closely match development of the capacity concept in the economic literature. Full capacity in a given industry is the level of output that can be reached under normal operating conditions. Measures of full capacity are variously defined as the minimum point of the long-run average cost curve, the full-input point on an aggregate production function, or a bottleneck point in a general equilibrium system (Klein and Long 1973).

Capacity Utilization

Capacity utilization (u) is an efficiency measure. In this paper we define a fleet's capacity utilization to be the ratio of actual output (Y) divided by Y^*, the ability to produce (Winston 1974; Morrison 1986).

$$(1) \quad u = Y/Y^*$$

A modification of the basic capacity utilization equation is the inclusion of an element recognizing changes in productivity (Nishimizu and Page 1982; Berndt and Fuss 1986; Hulten 1986). We call this technical efficiency. Technical efficiency encompasses productivity changes such as learning by doing or improving managerial skill. For example, as fishermen gain experience they learn more about the fishing grounds and effective use of gear. Thus, a fisherman using the same equipment may increase his share of the total catch as his technical efficiency improves. A fleet's technical efficiency could decline with new management restrictions or with the entry of inexperienced fishermen.

Unlike other industries, where capacity utilization (u) is usually known, capacity utilization is not known in fisheries. Fleet output (Y) is recorded in landing statistics. In order to assess capacity utilization, a method must be developed to measure fleet capacity (Y^*).

TABLE 1. Oregon trawl fleet characteristics, 1976–85. — No data available.

Year	Number vessels	Average (Net tons)	Trips per vessel	Average days per trip	Average catch per trip (Net tons)
1976	76	35	28	3.3	2.7
1977	79	36	20	3.4	2.8
1978	104	43	22	3.6	3.5
1979	152	48	23	3.3	3.5
1980	168	47	18	3.2	2.7
1981	152	47	23	3.4	3.2
1982	186	50	24	3.6	3.6
1983	182	47	28	—	3.3
1984	162	46	25	3.3	2.9
1985	133	46	24	3.2	3.5

In general terms the concepts of number, size, technical efficiency, and time as components of capacity apply to all fisheries. However, each fishery requires a set of calculations that are unique to its particular circumstances because the method of fishing affects the calculation. The general approach used here for the Oregon bottom trawl fishery is applicable to other types of fisheries.

Oregon Bottom Trawl Fishery Example

We use ten years of data, 1976–85, to indicate fleet capacity changes in the Oregon bottom trawl fishery. The fishery targets groundfish — round (primarily *Ophiodon elongatus*, *Anoplopoma fimbria*, and *Gadus macrocephalus*), rockfish (*Sebastes* spp. and *Sebastolobus alascanus*), and flatfish (mainly *Microstomus pacificus*, *Parophrys vetulus*, *Eopsetta jordani*, *Atheresthes stomias*, and *Glyptocephalus zachirus*). For most fishermen the groundfish fishery is full-time, "You do bottom fishing all year round, as you need to; it's not a fill-in fishery." (Dewees and Hawkes 1988).

Bottom trawl gear includes both "mud" gear for trawling on sandy and muddy ocean bottoms, and "roller" gear used to trawl over rocky bottoms. The modal bottom trawl vessel in this data set is 65 feet, 365 horsepower, and 57 net tons. During the 10-yr period the number of vessels fishing each year varies between 76 and 186 (Table 1). Fishermen also switch to other fisheries. Some trawlers catch crab (*Cancer magister*) in the winter, shrimp (*Pandalus jordani*) in the spring and summer, or participate in joint-venture fisheries for whiting (*Merluccius productus*) in the summer or fall. Vessels fish in Alaska, California, or Washington waters when opportunities are better there.

The 10-yr data set tracks the participation of 360 vessels. Data on vessel characteristics come from United States Coast Guard (U.S.C.G.) vessel documentation records, National Marine Fisheries Service inventories, Oregon Department of Fish and Wildlife records, and survey data collected from fishermen. Vessel characteristics include horsepower, length, and net tons for 98% of the vessels. The various data sources do not always agree on these vessel characteristics. In addition, upgrading vessels may change one or more dimension. We use the most recent information available, and where differences exist we verify each of the three characteristics by consulting the most recent U.S.C.G. information available. Therefore, the horsepower, length, and net ton figures used for all 10 yr are the most currently verified information. Due to constant

upgrading, this creates a bias toward overestimating the fleet capacity in earlier years.

To represent capacity, we chose net tons. This is a volume measure expressed in units of 100 cubic feet. The definition of net (register) tonnage used by the U.S.C.G. is the remainder after deducting from the gross tonnage of the vessel the tonnage of crew spaces, master's accommodations, navigation spaces, allowances for propelling power, and other noncargo space uses (U.S.C.G. 1981). Contacts with processors and fishermen indicate that each net ton, e.g. each 100 cubic feet, equates, on average, to a carrying capacity of 4,000 pounds of fish. Flatfish pack at slightly higher weights per net ton, while round fish weigh slightly less. This weight conversion of 4,000 pounds of fish per net ton assumes only fish are packed in the hold. Most bottom trawl vessels ice their fish, and the volume of ice would reduce space for fish by an estimated one-half (R. Young, F/V City of Eureka, CA; M. Thomas, Eureka Fisheries, Inc., CA, pers. comm.).

Net tons is highly correlated with horsepower ($r = 0.71$) and length ($r = 0.78$). Using 4,000 pounds of catch as the weight equivalent of a net ton gives the maximum hold capacity per vessel.

Number and Size Capacity Measures

Since the real value of measuring capacity is to assess its change over time, capacity is most meaningful when compared with a particular time period. To determine change in fleet number capacity (ΔNCap) for any year (t_i) from the base period (t_0), we subtract the number of vessels in the current year from the number in the initial year and multiply by the tonnage in the initial year.

$$(2)\quad \Delta\text{NCap}(t_i) = [\text{Vessels}(t_i) - \text{Vessels}(t_0)] \times [\text{Avg NT}(t_0)]$$

This equation indicates the addition to the initial capacity from an increase in the number of vessels. It looks only at the change in capacity due to changing the number of vessels. To control for size, each vessel is added at the same size as the average vessel in the initial time period.

The change in size aspect of capacity (ΔSCap) indicates how increases in vessel size affect fleet capacity. We subtract the average tonnage for the current year from the average tonnage for the initial year and multiply by the current total number of vessels.

$$(3)\quad \Delta\text{SCap}(t_i) = [\text{Vessels}(t_i] \times [\text{Avg NT}(t_i) - \text{Avg NT}(t_0)]$$

Technical Efficiency Measure

The technical efficiency (TEff) aspect of capacity accounts for the aggregate effect of how well people use their fishing equipment. Innovative fisherman introduce technical change to the fleet. Dewees and Hawkes (1988) found that trawlers adopted new technology when it met their needs and fit with their available resources. For example, those who adopted midwater trawling came more often than nonadopters from fishing families who could pool financial resources to invest in the expensive vessels and electronics required for this type of fishing. Opinion leaders validate new technical ideas for the rest of the fleet (Dewees and Hawkes 1988). As fishermen observe and share what they now about the effectiveness of gears, new fishing techniques, successful fishing strategies, and the best fishing areas, the collective knowledge of the fleet increases. This knowledge improves the overall technical efficiency of the fleet (Stephenson 1980).

As the technical efficiency of the fleet improves, more fish are caught with the same number and size capacity. Increases in technical efficiency compound existing capacity. It improves number plus size capacity by the multiple of the improvement in technical efficiency. We set technical efficiency at 1.0 for the base period. If all fleet members continue fishing in subsequent years and their experience increases, their technial efficiency improves. Assume that the technical efficiency of the fleet increases at 10% per year. After 7 yr the technical efficiency component of capacity doubles, and the multiplier changes from 1.0 at t_0 to 2.0 at t_7. In most fisheries there is continual entrance and exit. If those who take most of the catch stay in the fishery, the overall technical efficiency in the fishery should increase. If substantial numbers enter the fishery, then technical efficiency may decline until new fishermen learn the needed skills.

The technical efficiency contribution to fleet capacity growth is the most difficult to measure. Many factors influence technical efficiency. Some of these such as weather, variability in the availability of fish stocks (Charles 1983; Robarts et al. 1989), market conditions, regulations, competition with other gear types for fish and ocean space, and the addition of more trawlers are beyond the control of each fisherman. These are background factors. Factors such as effectiveness of specialized equipment, knowledge about the fishing environment, and ability of the crew are under the control of each vessel captain.

Measuring technical efficiency requires controlling for the effects of these factors. The best way to measure technical efficiency is to follow a sample of fishermen. Our landings data do not tell who landed the fish; therefore, following individuals was not possible. We estimate technical efficiency by comparing changes in a selected set of vessels.

Two groups of vessels provide an estimate of the technical efficiency. The first is 25 vessels which fished all 10 yr. Since this number is the same each year in terms of number and net tonnage, we assume that any trend in capacity utilization is due to improved technical efficiency. Those which fished all 10 yr are subject to the same background factors of weather, stock variability, market conditions, regulation, and competition. For this reason, the average capacity utilization of these 25 vessels is not the best indicator of improved technical efficiency. Instead we look at the best vessel's capacity utilization each year. The annual best capacity utilization in this group of 25 vessels shows a general increase from a low of 18% capacity utilization in 1976 to a maximum of 31% in 1983. There is a general increasing trend from 1976 to 1985.

A weakness in using the 25 vessels fishing all 10 yr is that the same person did not remain as captain on each of these 25 vessels all 10 yr. Technical efficiency may be held down by new fishermen entering the fishery. Assuming that the effects of changing vessels are random, then the pattern of change in capacity utilization indicates improvement in technical efficiency. A regression of the 10 annual maxima gives an increase in technical efficiency of 5.6% per year. The regression is significant ($F = 15.3$, $P \leq 0.004$).

The second technical efficiency estimate comes from following the standard vessel through the 10-yr period. This is the modal vessel of 65 feet, 365 horsepower, and 57 net tons. To get an adequate number of similarly sized vessels, we used all those in the 57–59 net ton range. Capacity utilization in this group increased from 7% in 1976 to 19% in 1982 and then dropped back to 16% in 1985. The pattern of the regression

TABLE 2. Relative importance of number, size, and technical efficiency in the Oregon trawl fleet capacity, 1976–85, in net tons.

Year	Initial capacity	Number capacity	Size capacity	Technical efficiency	Fleet capacity
1976	2660	0	0	0	2660
1977	2660	105	47	56	2869
1978	2660	980	874	182	4696
1979	2660	2660	1976	447	7743
1980	2660	3220	1982	648	8511
1981	2660	2660	1809	742	7871
1982	2660	3885	2786	1177	10509
1983	2660	3710	2275	1285	9930
1984	2660	3045	1777	1284	8766
1985	2660	1995	1530	1207	7391

shows an upward trend of 8.1% per year. The F-value of the regression is 4.9 ($P \leq 0.06$).

These two estimates suggest a pattern of increasing technical efficiency in the bottom trawl fleet. The 6–8% range of the annual increase in capacity utilization represents the maximum growth in technical efficiency. This was the rate at which the very best fishermen improved their technical efficiency. The whole fleet could not be expected to improve at this rate. Stephenson (1980) found 11% of the fishermen were "innovators."

Because the fishery is open access and there are strong competitive pressures, we estimate that from the 11% of the fishermen who initiate technical change roughly 25–35% of the innovative improvement in capacity utilization reaches the whole fleet each year. This gives a 2% annual rate of increase in technical efficiency for the fleet as a whole. A 2% annual increase from 1976–85 is twice that estimated by Robarts et al. (1989) for the period 1981–88. Robarts et al. measure total factor productivity. Because they control for vessel number and size, the rate of change in total factor productivity equates to the rate of change in technical efficiency. Robarts et al. acknowledge that resource abundance affects the measure of total factor productivity. Total factor productivity varied considerably during the 1980's. It declined in 1982 and 1983, grew for 1984–86, dropped in 1987, and rose again in 1988. Since the bottom trawl fleet rapidly modernized after 1976, the 2% rate of technical efficiency improvement for 1976–85 is reasonable.

Technical efficiency is cumulative, it compounds at 2% per year, causing the technical efficiency multiplier to increase from 1.0 the first year to 1.20 in the tenth year. Because of reductions of both number and size capacity during 1983, 1984, and 1985, the increase in technical efficiency reduces some of these overall capacity losses. Including technical efficiency, the capacity equation becomes

$$(4)\quad \text{Fleet Cap}(t_i) = [\text{Cap}(t_0) + \Delta\text{NCap}(t_i) + \Delta\text{SCap}(t_i)] \times [\text{TEff}(t_i)]$$

This gives an estimate of the dockside fishing capacity (Table 2), which is comprised of the number of vessels in the fleet, their size, and the technical efficiency with which they are fished.

Oregon bottom trawl fleet capacity quadrupled during the period 1976–82 (Table 2). The primary factor driving the increase was a more than doubling of the number of vessels. For the change in fleet capacities over the 10-yr period, number capacity accounts for 42%. The change in hold size capacity was the next most important contributor with 32% of the increase. The magnitude of change in size capacity between 1976 and 1982 equaled the annual fleet capacity in 1976. The size increase occurred very rapidly between 1977 and 1979. In this period vessel size increases appear to have been largely in response to the expectation of new fishing opportunities provided by the passage of the Magnuson Fishery Conservation and Management Act and by the ready availability of vessel financing. In the early 1980's, larger vessels entered the fishery to fish for widow rockfish (*Sebastes entomelas*) and Pacific whiting (*Merluccius productus*). Technical efficiency accounted for 26% of the fleet capacity increase from 1976–85.

Annual Comparisons of Fleet Capacity

Measures of industrial capacity typically look at annual differences. In a fishery, the unit of time accounting will vary between different fishing activities. A time unit common to most fisheries is the trip. Catches are reported by trip, and a trip is the smallest time unit during which the vessel hold is filled. The annual capacity of fishing vessels has to be understood in terms of the number of times vessels can fill their holds during the year. Depending on the fishery and on vessel size, trips vary in length and number per year. Large vessels may make a few long trips each year, while small vessels make many short trips. Catch limits imposed by regulation may also limit the number of trips. Determining the number of trips the vessels in a fleet can take each year often is quite complex.

Our data are records of each fishing trip made by all vessels landing in Oregon between 1976–85. Trips are the only consistent index of catch rate used for the full 10-yr period. A trip is also the smallest time period for filling the vessel hold, and hold size is our measure of size capacity.

To get a measure of annual capacity, fleet capacity (Eq. 4) is multiplied by the potential number of trips the average trawl vessel could make annually. Trips vary in length from one to 13 d. The average length of trips showed no pattern of change over the period of our data, ranging between 3.2 and 3.6 d (Table 1). The Pacific Fishery Management Council has adopted a management policy of maintaining the bottom trawl fishery for the entire year. With the exception of late fall closures for selected species, the full annual fishing season remained in place during the 10-yr period of our analysis.

The average number of bottom trawl trips ranged from a low of 18 in 1980 to almost 28 in 1976 and 1983 (Table 1). Trips averaged 3.4 d during the 10-yr period. This means the average vessel fished 61–95 d a year in the bottom trawl fishery. We conducted a survey of 25% of the Oregon bottom trawl fleet in 1986. Interviews with vessel owners revealed the average vessel spent 90 d trawling and made 26 trips in 1986. The survey sample was biased toward more productive vessels, which fished more often. The 1976 and 1983 average of 28 trips per year is a reasonable estimate for the largest number of trips vessels in the fleet would make in a year.

Equation 5 gives the annual fleet capacity.

$$(5)\quad \text{Ann Fleet Cap}(t_i) = \text{Fleet Capacity}(t_i) \times \text{Trips}(t_i)$$

Table 3 shows annual fleet capacity grew to almost 300 000 net tons by 1982 and then declined to just over 200 000 net tons in 1985.

Fleet Capacity Utilization

To determine the fleet capacity utilization in a year, we divide the annual fleet catch by the annual fleet capacity. For any particular year t_i

TABLE 3. Annual Oregon trawl fleet capacity utilization, 1976–85.

Year	Annual fleet capacity[a]	Annual catch capacity in NT	Utilization in % catch/capacity
1976	74480	6258	8.4
1977	80322	5235	6.5
1978	131487	7958	6.1
1979	216792	11389	5.3
1980	238294	9356	3.9
1981	220382	11326	5.1
1982	294240	15810	5.4
1983	278051	16233	5.8
1984	245448	11650	4.7
1985	206949	11612	5.6

[a]Fleet capacity from Table 2 times 28 trips.

$$(6)\quad \text{Fleet Cap Utilization}(t_i) = \text{Fleet Catch}(t_i)/\text{Fleet Cap}(t_i)$$

Fleet capacity utilization represents the ratio of the fleet's actual catch to its catching capability. Fleet capacity utilization varies from year to year with weather, market, regulatory, and other factors that affect the ability to catch fish.

Table 3 shows fleet capacity utilization was 8.4% in the base year, 1976. This sounds very low. The low value is partly due to the way we calculate capacity utilization. Fleet capacity utilization must be viewed in relative terms for the following reasons.

- Few trips land a full load.
- Ice in the hold to keep fish cold reduces the amount of fish the vessel can carry by as much as 50%. Adjusting for the average amount of ice used to store fish would reduce the size aspect of capacity.
- For both safety and comfort reasons vessels are often designed to be larger than the minimum size needed. For example, winter fishing requires larger vessels to contend with heavy weather conditions. Also, fishing in Alaska requires sturdier vessels than fishing in Oregon.
- The prestige of owning a large vessel is important to some fishermen (Dewees and Hawkes 1988).
- The fact that the West Coast market for groundfish is predominantly a fresh fish market constrains trip length.
- Trips end early due to equipment failure, weather conditions, and other unexpected events.
- Limits on certain species also require ending a trip before the hold is full.
- To maintain the flexibility to switch to other fisheries such as midwater trawl, joint venture, or crab, vessels may be larger than optimum for bottom trawling alone.
- A fishing vessel is a long-term investment. The vessel must be sized to accommodate new technologies.
- Participation in other fisheries takes time away from trawling.
- Federal tax incentives for fishermen during the late 1970's and early 1980s encouraged fishermen to build larger vessels.
- Fishermen are opportunistic. They do not want to be too small to take advantage of a particularly good fishing opportunity (Carter 1981; Herrick 1980).

All these factors lead to designing vessels with larger capacity than would be required considering only operational efficiency for a single fishery.

Between 1976 and 1982, fleet capacity more than tripled in the Oregon bottom trawl fishery as new and larger vessels entered the fishery. Elimination of foreign fishing created the expectation of large domestic availability of fish. West coast groundfish landings rose by 50% during this period (Pacific Fishery Management Council 1989). The imbalance between capacity growth and total catch resulted in fleet capacity utilization dropping by over half to a low in 1980 of 3.9%. Capacity peaked in 1982. Capacity utilization improved from 1981 to 1983 as large widow rockfish catches helped fleet capacity utilization rebound to 5.8%.

The 1982 recession caught the fishery in a cost-price squeeze — high interest rates, high fuel prices, and escalating insurance rates combined with low ex-vessel prices for groundfish. In addition, other options such as midwater trawling, joint venture opportunities, and fishing in other areas, primarily Alaska, reduced the number of vessels participating in the Oregon bottom trawl fleet. From 1982 to 1985 bottom trawl fleet capacity declined 30%. By 1984, limits placed on widow rockfish and other rockfish species curtailed groundfish catch. In 1984 and 1985, total catch was 25% below the 1983 level. Fleet capacity utilization in 1984 dropped by 20% and would have been even lower if 10% of the vessels had not left the bottom trawl fleet to pursue fishing opportunities elsewhere — primarily joint venture fisheries in Alaska (Robert Demory, Oregon Department of Fish and Wildlife, pers. comm.; trawler interviews). The number fishing declined further in 1985 and fleet capacity utilization rose.

Potential Capacity

The Oregon bottom trawl fishery is not the only one in which these fishermen are active. The decrease in fleet capacity after 1982 resulted, in part, from better opportunities elsewhere. This departure of vessels to other fisheries means that in addition to existing fleet capacity, there is a potential capacity which may easily return to the bottom trawl fleet.

Potential capacity is an estimate of the capacity likely to return to a fishery when opportunities elsewhere diminish. Assume half the 3,100 net ton capacity leaving the Oregon trawl fishery after 1982 went to Alaska or other fisheries from which they could easily return to the bottom trawl fleet. These vessels represent a potential capacity of 1,550 net tons, 21% of the 1985 total fleet capacity of 7,391 net tons. Looking at capacity from a multi-fishery perspective gets very complex. The purpose here is to illustrate that fleet capacity does not necessarily disappear. Capacity may merely be added to another fishery and return when conditions there change. Capacity may be retained in a fishery through foreclosure sales which reduce costs to new entrants.

Implications for Fishery Management

Fishery management is shifting from fleet controls on catch and effort to individual catch controls. Our development of capacity utilization shows that the dynamics of change in the fleet quota controlled the Oregon bottom trawl fishery. For fisheries using individual quota controls, capacity utilization will be a critical measure for determining whether the market for quotas is effectively allocating effort and reducing the race for fish. Regardless of whether fleet or individual controls are used, management to reduce overcapitalization should result in better capacity utilization ratios.

Capacity utilization can be a general indicator of what may be happening to economic efficiency. If capacity utilization is declining, this indicates decreased economic efficiency, except in those cases where higher prices are paid for small volumes

of well handled fish. By the same token, improved capacity utilization generally shows improved economic efficiency.

Identifying the elements of fleet capacity also indicates the management alternatives available for controlling fishing effort. Our analysis demonstrates the kinds of controls which have the most impact. The capacity formula (number plus size multiplied by the technical efficiency multiplied by the time spent fishing) highlights the effort control options available to fishery managers.

Fishery management typically begins with controls on the temporal aspect of capacity, the length of time fished. Open and closed seasons set the time for fishing. As effort grows, fishing seasons are shortened. The obvious problem with temporal controls is that they lower capacity utilization because equipment lies idle for a portion of the year. In fact, shortened seasons can stimulate capacity increase, since the hold size of a vessel in a short season is critical to the level of output.

A strategy for managing number and size capacity together is to base limitations on the number of net tons. This, however, does not deal with either the frequency of fishing or the technical efficiency of those fishing. Fishermen usually are innovative enough to skirt the effects of most controls and still increase their technical efficiency.

All capacity growth is not detrimental to a fishery. In the building stages of a fishery, capacity is often below what is needed to attain the allowable catch. As capacity grows, a point is reached when more fleet capacity is available than needed to take the annual allowable catch. Thus, when a fishery is developing, capacity needs to grow. Once the fishery matures, excess capacity becomes a problem.

If management is to control capacity growth using an effort limitation program, the greatest impact usually comes from limiting the number of vessels. Because capacity is more than number, this policy can only be marginally successful. Controlling the number of vessels fishing creates incentives to increase the other elements of capacity — size, technical efficiency, and the frequency of fishing (Hanna and Silverthorne 1987). In addition, other factors enter into management decisions. Political pressures keep the maximum number of people fishing. Managing size could allow more people to participate, but may prevent vessels from realizing economies of scale.

Individual transferable quotas are a way to use market mechanisms to control all aspects of capacity. However, catch management through individual quotas will not be a panacea for fisheries. Both theoretical and empirical investigations have pointed out some weaknesses of individual transferable quotas. Copes (1986) enumerates within-fishery interactions which weaken many of the potential benefits of an individual quota system. Using data from New Zealand, Dewees (1989) shows that individual transferable quotas encounter many of the same problems as trip quotas or season quotas, namely poor reporting, bycatch, and high grading. Under individual transferable quotas the market for particularly high-value species might actually increase the application of effort to the resource as fishermen try to take the largest amount of fish from which to select the few with highest market value. Similarly, if a small quota is set for one species caught in an assemblage, attainment of the single species quota may shut down the fishery on all species in the assemblage. This situation may create incentives to again "race for the fish" before the fishery is closed. Despite these potential problems, individual quotas do appear to improve ecnomic efficiency (Dewees 1989).

The concept of capacity highlights the alternatives available for fishery management and the reasons why some alternatives do not achieve their desired end. This perspective on fleet capacity also shows that no one management measure will effectively control capacity growth. The number of vessels, their size, the technical efficiency of those fishing, and the time spent fishing all contribute to capacity utilization. In situations where capacity utilization is declining, fish stocks will be under pressure from intensified fishing. Capacity utilization is a useful indicator of effective fishery management. Improved management should improve capacity utilization.

Acknowledgements

This research was supported in part by the citizens of Oregon and the Oregon State University Sea Grant College Program under grant No. NA85AA-D-SG095 from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce. We appreciate helpful comments from Tony Charles, David Reid, James Golden, Joe Easley, Sam Herrick, Richard Young, and anonymous manuscript reviewers.

References

ADASIAK, A. 1978. The Alaskan experience with limited entry, *In* R. B. Rettig and J. C. Ginter [ed.] Limited entry as a fishery management tool. University of Washington Press, Seattle, WA.

BERNDT, E. R., AND M. A. FUSS. 1986. Productivity measurement with adjustments for variations in capacity utilization and other forms of temporary equilibrium. J. Econometrics 33(1/2): 7–29.

CAMPBELL, B. A. 1972. Limited entry in the salmon fishery; the British Columbia experience. Pacific Sea Grant Advisory Program 6: 13 p.

CARTER, C. N. 1981. Multi-fishery activity in Oregon commercial fishing fleets: an economic analysis of short-run decision-making behavior. Ph.D. dissertation, Oregon State University, Corvallis, OR. 200 p.

CHARLES, A. T. 1983. Optimal fisheries investment under uncertainty. Can. J. Fish. Aquat. Sci. 40: 2080–2091.

COPES, P. 1986. A critical review of the individual quotas as a device in fisheries management. Land Econ. 62(3): 278–291.

DEWEES, C. M. 1989. An assessment of the implementation of individual transferable quotas in New Zealand's inshore fishery. N. Am. J. Fish. Manage. 9: 131–139.

DEWEES, C. M., AND G. R. HAWKES. 1988. Technical innovation in the Pacific coast trawl fishery: the effects of fishermen's characteristics and perceptions on adoption behavior. Human Organ. 43: 224–34.

DURANTI, A. 1985. Sociocultural dimensions of discourse, p. 193–230. *In* T. A. van Dijk [ed.] Handbook of discourse analysis. Academic Press, New York, NY.

HANNA, S., AND W. SILVERTHORNE. 1987. Interaction with existing management measures, *In* D. D. Huppert [ed.] Limited access alternatives for the Pacific groundfish fishery. U. S. Department of Commerce, NOAA Tech. Rep. NMFS 52. 45 p.

HERRICK, S. 1980. An economic analysis of the long run decision-making behavior of Oregon otter trawl fishermen Ph.D. dissertation, Oregon State University, Corvallis, OR. 137 p.

HULTEN, C. R. 1986. Productivity change, capacity utilization, and the sources of efficiency growth. J. Econometrics 33(1/2): 31–50.

KEEN, E. A. 1973. Limited entry: the case of the Japanese tuna fishery, *In* A. A. Sokoloski [ed.] Ocean fishery management: discussions and research. U. S. Department of Commerce, NOAA Tech. Rep. NMFS CIRC-371.

KLEIN, L. R., AND V. LONG. 1973. Capacity utilization: concept, measurement, and recent estimates. Brookings Papers on Economic Activity 3: 1973.

MEANY, T. F. 1978. Restricted entry in Australian fisheries, *In* R. B. Rettig and J. C. Ginter [eds.] Limited entry as a fishery management tool. University of Washington Press, Seattle, WA.

MORRISON, C. J. 1985. Primal and dual capacity utilization: an application to productivity in the U. S. automobile industry. J. Bus. Econ. Stat. 3(4): 312–324.

1986. Productivity measurement with non-static expectations and varying capacity utilization. J. Econometrics 33(1/2): 51–74.

MULKAY, M. 1985. The word and the world: explorations in the form of sociological analysis. Allen and Unwin, London, UK. 263 p.

NISHIMIZU, M., AND J. M. PAGE, JR. 1982. Total factor productivity growth, technological progress and technical efficiency change: dimensions of productivity change in Yugoslavia, 1965–78. Econ. J. 92(December): 920–936.

PACIFIC FISHERY MANAGEMENT COUNCIL. 1989. Status of the Pacific coast groundfish fishery through 1989 and recommended acceptable biological catches for 1990. Pacific Fishery Management Council, Metro Center Suite 420, 2000 SW First Avenue, Portland, OR. 49 p.

PEARSE, P. H., AND J. E. WILEN. 1979. Impact of Canada's Pacific salmon fleet control program. J. Fish. Res. Board Can. 36: 764–89.

ROBARTS, C., L. NGUYEN, AND D. SQUIRES. 1989. Productivity measurement in the Pacific Coast trawl fleet, 1981–1988. National Marine Fisheries Service, Southwest Fisheries Center Admin. Rep. LJ-89-25.

STEPHENSON, G. O. 1980. "Pushing for the highline": the diffusion of innovations in the Oregon otter trawl fishery. M.A. in Interdisciplinary Studies thesis, Oregon State University, Corvallis, OR. 91 p.

UNITED STATES COAST GUARD (U.S.C.G.). 1981. Merchant vessels of the United States. Vol. 2. U. S. Department of Transportation, Washington, D. C.

WINSTON, D. C. 1974. The theory of capital utilizatio and idleness. J. Econ. Lit. 12(4): 1301–1315.

Treatment of Data for Duplicated Gene Loci in Mixed-Stock Fishery Analysis

Robin S. Waples and Paul B. Aebersold

National Marine Fisheries Service, Northwest Fisheries Center 2725 Montlake Blvd. East, Seattle, Washington 98112, USA

Waples, R. S., and P. B. Aebersold. 1990. Treatment of data for duplicated gene loci in mixed-stock fishery analysis. Can. J. Fish. Aquat. Sci. 47: 2092–2098.

Isoloci (pairs of duplicated loci with a predominance of shared alleles of identical electrophoretic mobility) are common in salmonid fishes and present special problems for the analysis of mixed-stock fisheries. The method currently used to treat isolocus data (which assumes that allele frequencies at both loci are the same) leads to bias if the true frequencies (P, Q) are not identical. Using simulated mixtures drawn from populations with (1) hypothetical allele frequencies and (2) allele frequencies observed at four isolocus systems in samples of chinook salmon from the Fraser River system, we show that a better method is to use the maximum likelihood estimates ($\hat{P}$, $\hat{Q}$) of allele frequencies at the individual loci. In simulations in which the population frequencies at the two loci of an isolocus pair differed, use of ($\hat{P}$, $\hat{Q}$) resulted in less error in estimating the true mixture proportions than did use of a single average frequency, whereas the two methods produced very similar results if population frequencies were identical at both loci.

Les isoloci (paires de loci doubles avec prédominance d'allèles partagés de mobilité électrophorétique identique) sont courants chez les salmonidés et présentent des problèmes spéciaux pour l'analyse des pêches de divers stocks. La méthode actuellement employée pour traiter les données relatives aux isoloci (qui part de l'hypothèse selon laquelle les fréquences alléliques aux deux loci sont identiques) est une source d'erreurs lorsque les fréquences vraies (P, Q) ne sont pas identiques. À l'aide de mélanges simulés de populations (1) aux fréquences alléliques hypothétiques et (2) aux fréquences alléliques observées dans quatre (4) systèmes d'isolocus, dans des échantillons de saumon quinnat provenant du réseau du fleuve Fraser, nous montrons qu'il existe une meilleure méthode qui consiste à utiliser les estimations du maximum de vraisemblance ($\hat{P}$, $\hat{Q}$) des fréquences alléliques à chaque locus. Dans les simulations où les fréquences relatives à la population aux deux loci d'une paire d'isolocus étaient différentes, l'emploi de $\hat{P}$, $\hat{Q}$ a occasionné moins d'erreurs dans l'évaluation des proportions vraies des mélanges que l'utilisation d'une seule fréquence (fréquence moyenne), alors que les deux méthodes donnaient des résultats très semblables lorsque les fréquences relatives à la population étaient identiques aux deux loci.

Received January 26, 1989
Accepted May 10, 1990
(JA025)

Reçu le 26 janvier 1989
Accepté le 10 mai 1990

The Genetic Stock Identification method (GSI) is currently used to provide stock composition estimates for a number of mixed-stock fisheries of Pacific salmon, *Oncorhynchus* spp. (Utter et al. 1987b; Beacham et al. 1987; Washington Department of Fisheries 1988) and other salmonids (Marsden et al. 1989). The purpose of this paper is to present a modification in the treatment of certain genetic data that should increase precision of the method.

The basic data used in GSI are genotypic frequencies observed in a sample from the fishery and estimated from samples of potential source populations. Resolution of the mixture is possible if genotypic frequencies differ sufficiently among contributing populations. Maximum likelihood estimates of the relative contributions of individual populations are obtained by finding the set of composition estimates with the highest probability of producing the suite of genotypes observed in the mixture, given the frequencies of those genotypes estimated for the source populations. Because data from a number of loci are usually required to obtain reasonably accurate composition estimates, multilocus genotypes are routinely recorded for each fish.

A complication in the analysis is the difficulty in adequately sampling all possible multilocus genotypes in the source populations. For example, in diploid organisms three genotypes (AA, Aa, aa) can occur at a gene locus with two alleles (A, a); if ten such loci are considered, 3^{10} (over 59 000) different multilocus genotypes are possible. Samples generally available from source populations (typically 50–100 individuals) are far too small to provide adequate representation of all possible genotypes. For this reason, expected genotypic frequencies have often been substituted for those actually observed in the source population samples, the expected frequencies being those that would occur under conditions of Hardy–Weinberg equilibrium (Milner et al. 1981; Pella and Milner 1987). Because Hardy–Weinberg expected frequencies are simple functions of observed allele frequencies, it is easy to compute the probability of a given population producing any particular multilocus genotype. Provided that certain assumptions are not seriously violated (e.g. that mating is random and the loci assort independently), this approach is suitable for use with uncomplicated genetic systems in diploid organisms.

Salmonids, however, are ancestrally tetraploid, and although diploid expression has been restored for some genes (Allendorf and Thorgaard 1984), others remain duplicated and present special analytical problems. In particular, isoloci (pairs of duplicated loci with a predominance of shared alleles of identical

TABLE 1. Nine genotypes and the five electrophoretically detectable phenotypes for a pair of isoloci (L_1, L_2) with two alleles each (A, a). Unconditional expected genotyic frequencies make no assumptions regarding the allele frequencies (P, Q) of A at L_1 and L_2; conditional expected genotypic frequencies assume that $P = Q = \bar{P}$. Expected phenotypic frequencies are merely the sum of the expected frequencies for all genotypes having the same phenotype.

Genotype		Expected genotypic frequency		
L_1	L_2	Unconditional	Conditional on $P = Q = \bar{P}$	Phenotype[a]
AA	*AA*	P^2Q^2	$\bar{P}^4$	O
Aa	*AA*	$2P(1-P)Q^2$	$4\bar{P}^3(1-\bar{P})$	I
AA	*Aa*	$2P^2Q(1-Q)$		
aa	*AA*	$(1-P)^2Q^2$		
AA	*aa*	$P^2(1-Q)^2$	$6\bar{P}^2(1-\bar{P})^2$	II
Aa	*Aa*	$4PQ(1-P)(1-Q)$		
Aa	*aa*	$2P(1-P)(1-Q)^2$	$4\bar{P}(1-\bar{P})^3$	III
aa	*Aa*	$2Q(1-Q)(1-P)^2$		
aa	*aa*	$(1-P)^2(1-Q)^2$	$(1-\bar{P})^4$	IV

[a]Phenotypes are labeled according to the number of doses of the variant (a) allele.

electrophoretic mobility) represent a significant percentage of the genome in salmonids (Allendorf and Thorgaard 1984) and are routinely used in GSI analyses. For example, recent mixture analyses of chinook salmon, *O. tshawytscha* (Washington Department of Fisheries 1988), and lake trout, *Salvelinus namaycush* (Marsden et al. 1989) each used four pairs of isoloci in addition to several single locus systems.

The analytical problems posed by isoloci can be understood by use of a simple model. Consider a pair of isoloci (L_1, L_2), each segregating in a population for the same two alleles: a "common" allele (A) and a "variant" allele (a). Let P be the population frequency of A at L_1 and let Q be the frequency of A at L_2. In a sample of individuals from this population, nine two-locus genotypes are possible, and their expected frequencies in terms of P and Q are given in Table 1. The nine genotypes, however, do not all produce unique electrophoretic phenotypes; only five such phenotypes are possible, corresponding to the number of doses (0–4) of the variant allele a (Table 1). Because not all genotypes can be distinguished, it is not possible to determine sample allele frequencies for L_1 and L_2 separately by simple gene counting.

The inability to directly compute allele frequencies for the individual loci of an isolocus system creates a problem in GSI analyses. The difficulty discussed above in adequately sampling all genotypic combinations in the source populations are magnified for isoloci. Five different isolocus phenotypes are possible for even a two-allele system, and this number increases very rapidly for systems with three or more alleles. Clearly, it is important to be able to compute expected frequencies for the isolocus phenotypes. This can be done for any given values of P and Q, because expected phenotypic frequencies are merely the sum of the expected genotypic frequencies that produce the same phenotype (see Table 1). The problem, of course, is that P and Q are not known. A common solution has been to assume that P and Q are the same, in which case the appropriate estimate is the average allele frequency observed over both loci in the sample. This assumption is equivalent to treating the pair of isoloci as a single, tetrasomic locus. The estimated mean frequency, $\hat{\bar{P}}$, is computed by counting the total number of A alleles in the sample and dividing by the total number of alleles observed (four doses per individual). Assuming $P = Q = \bar{P}$, the probability of getting 0, 1, ... 4 doses of the variant a allele (i.e. the probability of phenotypes O, I, ... IV) is given by the terms of the binomial distribution (Table 1, column 3).

The problem with using a single mean allele frequency is that it leads to bias if frequencies at L_1 and L_2 are not in fact the same. This can be seen by comparing expected phenotypic frequencies conditional on $P = Q = \bar{P}$ with the unconditional expected frequencies, which do not make this assumption (Table 1). $\bar{P}$ gives the correct expected frequencies only if $P = Q$. Otherwise, use of $\bar{P}$ will always overestimate the frequencies of phenotypes O and IV, and in general, estimates for the other phenotypes will also be biased, although the sign of the deviation will vary with the actual frequencies P and Q. The magnitude of the bias from using $\bar{P}$ increases with the difference between the actual frequencies P and Q.

The solution to this problem is to use estimates of allele frequency at the individual loci, rather than a single average allele frequency ($\hat{\bar{P}}$), to compute the expected phenotypic frequencies. Maximum likelihood (ML) estimates of P and Q ($\hat{P}$, $\hat{Q}$) can be obtained from the isolocus phenotypes by the method described by Waples (1988). The advantage to using the ML estimates in GSI analyses is simple: $\hat{P}$ and $\hat{Q}$ are the allele frequencies with the highest probability of producing the array of phenotypes observed in the sample, so they are the appropriate values to use in computing expected phenotypic frequencies.

In this study, we use computer-simulated mixtures to show that if the population allele frequencies at a pair of isoloci differ, use of ($\hat{P}$, $\hat{Q}$) results in less error in estimating true mixture proportions than does the use of a single average frequency, whereas the two methods produce very similar results if the true population frequencies are identical at both loci.

Methods

Computer Simulations

Baseline data

Several different computer simulations were performed, but all shared some common features. First, a matrix of parametric allele frequencies (P, Q) was selected for five populations and four or five isolocus systems; in some simulations these allele frequencies were chosen to be the same at both loci of an isolocus pair, and in other cases they were allowed to differ. The initial simulations used hypothetical population allele frequencies; in other simulations, parametric allele frequencies were chosen to correspond to those observed in samples from actual salmon populations (see below). For each replicate in a simulation, Monte Carlo methods were used to draw baseline samples (generally 100 fish) for each population, using the parametric population allele frequencies and the equations in Table 1 to determine the probability of each isolocus phenotype. The different isolocus systems were assumed to be independent. After each baseline sample was chosen, the ML estimates $\hat{P}$, $\hat{Q}$ were computed for each isolocus system as described by Waples (1988); in this method, if the two estimates differ, the lower is arbitrarily designated $\hat{P}$. The ML estimates, or their average [$\hat{\bar{P}} = (\hat{P} + \hat{Q})/2$], were used as baseline frequencies in analyzing the mixture.

Mixture data

The mixture size was fixed at 500 fish, which were chosen in the same way as the baseline samples, after first determining the source population for each fish as follows. In each replicate, four random numbers [X_i, $i = 1, 4$; $0 < X_i < 1$] were selected and ordered by increasing size; with the addition of X_0 and X_5 (defined as 0 and 1, respectively), these numbers served as cutpoints defining the probability of origin from each source population. For each fish in the mixture, another random number Y ($0 < Y < 1$) was chosen, and the fish was drawn from population i if $X_{i-1} < Y < X_i$. As new X_i were chosen for each replicate, this method allowed the probability of origin from the different source populations to vary randomly over the course of the simulation (thus avoiding results specific to any particular mixture proportions) but ensured that the average representation of the source populations was nearly equal over all replicates.

Mixture analysis

The mixture analysis used a modified version of Milner et al.'s (1981) program, which uses the EM algorithm to estimate the contributions by individual populations. Each replicate mixture was analyzed by two methods that differed in treatment of the baseline allele frequencies. In the "old" method, which is currently used in most GSI applications, baseline allele frequencies were assumed to be the same at each locus of an isolocus pair, so phenotypic probabilities and likelihoods were calculated using $\hat{P} = (\hat{P} + \hat{Q})/2$, or the average of the ML estimates for the individual loci. In the "new" method, phenotypic probabilities were calculated using the ML estimates themselves. In the mixture analysis program, this simply required substitution of the equations in the second column of Table 1 for those in the third column in calculating probabilities of a particular phenotype arising from a particular source population.

Because the EM algorithm may not quite attain the exact maximum likelihood solution within a specified number of iterations, the analysis in each replicate was terminated when the difference in log-likelihood between successive iterations fell below 10^{-6} (the "tolerance" level) or when 1000 iterations had been completed, whichever came first. In each replicate, the mean difference (per population) between the true and estimated mixture proportions was computed for both the old and the new methods. In addition to this direct measure of performance, we also calculated the number of iterations required for convergence and the log-likelihood of the solution for both methods. In each simulation, overall mean values for the above quantities were computed by averaging over 1000 replicates.

Chinook Salmon Data

Actual population data considered here were taken from 23 samples of chinook salmon collected in 1986–88 from the Fraser River system and other rivers in British Columbia, Canada. The samples included wild-caught juveniles, wild-caught adults, and juvenile progeny of fish that had been captured in the wild and transported to the Birkenhead, Chilliwack, Clearwater, Eagle, Quesnel, Shuswap, and Spius Creek hatcheries for spawning and rearing. Methods used to collect, transport, and store these samples are described in Aebersold et al. (1987).

Procedures for horizontal starch gel electrophoresis, including buffer and stain recipes, are also described in Aebersold et al. (1987). Gene products were resolved at four isolocus systems using the following tissue-buffer combinations: aspartate aminotransferase (*AAT-1,2*) from muscle tissue on a TBE gel; isocitrate dehydrogenase (*IDH-3,4*) and malate dehydrogenase (*MDH-3,4*) from muscle tissue on a ACE7 gel; and phosphoglucomutase (*PGM-3,4*) from eye tissue on a TBCLE gel. Gel interpretation followed the guidelines outlined in Utter et al. (1987a). In resolving and scoring the *PGM-3,4* isolocus system, we used protocols developed by Boyd Bentley, University of California at Davis (pers. comm.).

Maximum likelihood estimates of allele frequency at the individual gene loci were computed using the method of Waples (1988). For simplicity, all isoloci were analyzed as two-allele systems. For *MDH-3,4* and *IDH-3,4*, this required combining two or more variant alleles into a single "variant allele" class in some populations. Chi-square goodness-of-fit tests (Waples 1988) were used to compare observed and expected phenotypic distributions at the new *PGM-3,4* isolocus system.

Although the simple model used here assumes two alleles at each locus, ML estimates can also be obtained for systems with multiple alleles (Waples 1988). (IBM-PC compatible computer programs to compute the ML estimates for systems with two and three alleles will be provided on request to those supplying a blank, formatted 3.5 or 5.25 in diskette.)

TABLE 2. Results of analyses of simulated mixtures drawn from five hypothetical populations. In each simulation, the mixture contained 500 fish and baseline sample size for each population was 100. P and Q are the parametric allele frequencies, which differed among populations but, within each population, were the same at each of five isolocus systems. Actual mixture proportions varied randomly among replicates (see text for discussion). Results shown are means over 1000 replicates for the "old" method for estimating baseline allele frequencies [using a single average frequency ($\hat{P}$) for both loci] and the "new" method [using the ML estimates ($\hat{P}$, $\hat{Q}$)].

	Simulation					
A) Parameters	1		2		3	
Population	P	Q	P	Q	P	Q
1	0.0	0.6	0.3	0.3	0.1	0.9
2	0.0	0.8	0.4	0.4	0.2	0.8
3	0.0	1.0	0.5	0.5	0.3	0.7
4	0.2	1.0	0.6	0.6	0.4	0.6
5	0.4	1.0	0.7	0.7	0.5	0.5
B) Results	Old	New	Old	New	Old	New
Error[a]	0.125	0.018	0.077	0.080	0.222	0.132
Iterations[b]	522	65	631	510	998	797
Log likelihood	−2958	−2182	−3594	−3598	−3163	−3078

[a]Mean difference between true and estimated mixture proportions over all five populations.

[b]Number of iterations required for EM algorithm to converge.

Results

Mixtures Using Hypothetical Population Allele Frequencies

Three different simulations were performed using hypothetical population allele frequencies; in each case, five stocks and five isolocus systems were used, allele frequencies within a stock were the same at all loci, and 100 fish were drawn for each baseline sample. In the first two simulations, average allele frequencies ($\bar{P}$) for all isoloci were 0.3, 0.4, 0.5, 0.6, 0.7 for populations 1–5, respectively (Table 2A). The two simulations differed in the true allele frequencies (P, Q) at the

individual loci: in simulation 1, P and Q differed maximally, whereas in simulation 2 the two loci had identical frequencies. These two simulations thus represent the extreme range of scenarios likely to occur in natural populations.

Results of these simulations (Table 2B) demonstrate the improved resolution provided by use of the ML estimates (the new method). In simulation 1, with true population allele frequencies substantially different at L_1 and L_2, the mean error in estimating mixture proportions for each stock was reduced by nearly an order of magnitude (mean error = 0.018 per population vs. 0.125 for the old method). The solution was also reached much more quickly with the new method (mean of 65 iterations of the EM algorithm vs. 522 for the old method), and the mean log-likelihood for the solution showed a huge increase (from -2958 to -2182) with use of the new method. By several important criteria, then, the new method was far superior to the old in analyzing the mixtures in simulation 1.

That the new method also performed well if the true population allele frequencies were the same at both loci is demonstrated by the results of simulation 2. In this case, resolution of the mixture was still possible because the average allele frequencies differed among populations, but precision was not as great as was the case when frequencies at the individual loci also differed. Conversely, the old method, which assumes that $P = Q$ at every isolocus, performed better than it did in simulation 1, when this condition was not met. The net result was that the mean error per population, number of replicates required, and log-likelihood were very similar for the two methods. Not surprisingly, given that the assumption that $P = Q$ was met, the old method had a slightly lower mean error in simulation 2 (0.077 per population vs. 0.080 for the new method) and a slightly higher average log-likelihood for the solution, but the new method typically reached the solution more quickly (mean of 510 replicates vs. 631 for the old method) and provided estimates with less mean error in almost half (47%) of the replicates (R. S. Waples, unpubl. data).

The new method also had a clear advantage in simulation 3, in which all five populations shared the same average allele frequency ($\bar{P} = 0.5$) at all isolocus systems, but the populations varied in the extent to which P and Q differed (Table 2A). In such a situation, the old method has no basis at all for resolving the mixture, as is indicated by the large average error in estimating true mixture proportions (0.222 per population) and the failure of the EM algorithm to converge below the tolerance level within 1000 iterations in almost every replicate (Table 2B). Again, by all three criteria the new method performed much better than the old method, demonstrating that some resolution is possible even if average isolocus allele frequencies do not differ among populations. The composition estimates, however, were not nearly as precise (mean error 0.132 per population) as they were in simulation 1, in which $\bar{P}$ also differed among populations.

Analysis of Isolocus Data from Natural Populations

The simulated mixtures described next used frequencies observed in real populations, including data for an isolocus sytem (*PGM-3,4*) not previously reported in chinook salmon. We screened *PGM-3,4* in 40–80 fish from each of 23 populations in British Columbia (P. B. Aebersold and C. K. Shiflett, unpubl. data). In many samples, individuals with three or four doses of the less common allele were found, indicating that both loci must be segregating in this isolocus system. Average allele frequencies varied considerably among populations, from $\bar{P} = 0.971$ in the Babine River sample to $\bar{P} = 0.563$ at Wannock River, indicating that this isolocus system is potentially a very valuable one for resolving stock mixtures.

To help evaluate the reliability of the *PGM-3,4* data, we used the method described by Waples (1988) to perform goodness-of-fit tests comparing observed and expected phenotypic distributions. These tests are analogous to the familiar test of Hardy–Weinberg genotypic proportions at disomic loci and provide a means of testing for agreement with predictions of a simple genetic model (random mating, independent assortment of the two loci in an isolocus pair, and selective neutrality). The test is also useful in screening for scoring errors, which are important to consider with isoloci because some phenotypes must be distinguished on the basis of relative intensities of the same set of electrophoretic bands (Allendorf et al. 1975; Waples 1988). The possibility of scoring errors is particularly relevant for PGM, which is a monomeric enzyme and does not produce the intermediate, heterodimeric bands that are useful in scoring isolocus phenotypes for the dimeric enzymes AAT, IDH, and MDH.

For a diallelic locus, the goodness-of-fit test has two degrees of freedom [5 phenotypic classes − 1–2 parameters estimated ($\hat{P}$, $\hat{Q}$)]. Based on a critical $\chi^2_{[2]}$ value of 5.99 for $\alpha = 0.05$, a significant departure from expected phenotypic proportions was found in only one sample (Willow River; observed numbers of phenotypes O–IV = 17, 11, 3, 5, 0; expected numbers = 13.2, 15.1, 6.5, 1.2, 0.1; $\chi^2 = 15.71$; $P < 0.001$). This is a highly significant result, and would be expected to occur less than 5% of the time by chance even when Cooper's (1968) method is used to account for multiple independent tests ($P < \alpha/23 = 0.002$). Examination of a photograph of the gel containing the *PGM* data for the Willow sample suggested that scoring errors may have been responsible for the poor fit. The revised phenotypic distribution based on the photograph (17, 13, 4, 2, 0 individuals for phenotypes O–IV, respectively) is in much better agreement with expectations ($\chi^2 = 2.47$, $P > 0.05$) based on the revised ML estimates ($\hat{P} = \hat{Q} = 0.813$). In contrast, a blind rescoring of five other samples from the Fraser River system yielded phenotypic distributions that were in perfect agreement with, or differed by no more than one or two individuals from, the original data. In this example, therefore, the goodness-of-fit test proved useful in identifying a sample that deserved closer scrutiny.

We tentatively regard the test results as support for our scoring and interpretation of the *PGM-3,4* isolocus system, although we recognize the need for larger samples and more conclusive evidence that the genetic model is correct (breeding studies would be very useful in this respect).

Mixtures Using Fraser River Data

The final set of mixture analyses used as population parameters allele frequencies found in actual samples of chinook salmon from the Fraser River system. Recently, our laboratory has used baseline data from these and other Fraser River samples to analyze mixtures collected in a test fishery near the mouth of the river (G. B. Milner and D. J. Teel, unpubl. data), the goal being to determine the migration timing of the various stocks. Here, data for four isolocus systems (*AAT-1,2*; *IDH-3,4*; *MDH-3,4*; *PGM-3,4*) were used, and we selected five of the 23 populations discussed above to obtain a representative range of allele frequencies for these loci (Table 3). The Fraser

TABLE 3. Phenotypic distributions for four pairs of isoloci in five samples of chinook salmon from the Fraser River drainage. Phenotypic designations indicate the number of doses of the variant allele expressed. Estimated population allele frequencies [($\hat{P}$, $\hat{Q}$) or $\hat{\bar{P}} = (\hat{P} + \hat{Q})/2$] were used in simulations to obtain the results shown in Table 4.

	AAT 1,2 Phenotypes					*IDH 3,4* Phenotypes					*MDH 3,4* Phenotypes					*PGM 3,4* Phenotypes				
Sample	O	I	II	III	IV	O	I	II	III	IV	O	I	II	III	IV	O	I	II	III	IV
Birkenhead River	28	11	0	0	0	35	3	2	0	0	40	0	0	0	0	12	22	5	1	0
	$\hat{P} = 0.859$; $\hat{Q} = 1.0$					$\hat{P} = 0.956$; $\hat{Q} = 0.956$					$\hat{P} = 1.0$; $\hat{Q} = 1.0$					$\hat{P} = 0.617$; $\hat{Q} = 0.945$				
	$\hat{\bar{P}} = 0.929$					$\hat{\bar{P}} = 0.956$					$\hat{\bar{P}} = 1.0$					$\hat{\bar{P}} = 0.781$				
Eagle River	80	0	0	0	0	53	24	0	0	0	71	9	0	0	0	15	18	5	2	0
	$\hat{P} = 1.0$; $\hat{Q} = 1.0$					$\hat{P} = 0.844$; $\hat{Q} = 1.0$					$\hat{P} = 0.944$; $\hat{Q} = 1.0$					$\hat{P} = 0.788$; $\hat{Q} = 0.788$				
	$\hat{\bar{P}} = 1.0$					$\hat{\bar{P}} = 0.922$					$\hat{\bar{P}} = 0.972$					$\hat{\bar{P}} = 0.788$				
Pitt River	70	8	0	0	0	76	4	0	0	0	66	14	0	0	0	35	5	0	0	0
	$\hat{P} = 0.949$; $\hat{Q} = 1.0$					$\hat{P} = 0.975$; $\hat{Q} = 1.0$					$\hat{P} = 0.912$; $\hat{Q} = 1.0$					$\hat{P} = 0.937$; $\hat{Q} = 1.0$				
	$\hat{\bar{P}} = 0.974$					$\hat{\bar{P}} = 0.988$					$\hat{\bar{P}} = 0.956$					$\hat{\bar{P}} = 0.969$				
Walker Creek	80	0	0	0	0	79	0	0	0	0	63	17	0	0	0	13	20	7	0	0
	$\hat{P} = 1.0$; $\hat{Q} = 1.0$					$\hat{P} = 1.0$; $\hat{Q} = 1.0$					$\hat{P} = 0.894$; $\hat{Q} = 1.0$					$\hat{P} = 0.575$; $\hat{Q} = 1.0$				
	$\hat{\bar{P}} = 1.0$					$\hat{\bar{P}} = 1.0$					$\hat{\bar{P}} = 0.947$					$\hat{\bar{P}} = 0.788$				
White Horse Bluff	80	0	0	0	0	75	4	0	0	0	75	5	0	0	0	3	17	16	4	0
	$\hat{P} = 1.0$; $\hat{Q} = 1.0$					$\hat{P} = 0.975$; $\hat{Q} = 1.0$					$\hat{P} = 0.969$; $\hat{Q} = 1.0$					$\hat{P} = 0.344$; $\hat{Q} = 0.893$				
	$\hat{\bar{P}} = 1.0$					$\hat{\bar{P}} = 0.987$					$\hat{\bar{P}} = 0.984$					$\hat{\bar{P}} = 0.619$				

TABLE 4. Results of analyses of simulated mixtures drawn from five populations with allele frequencies identical to those in samples of chinook salmon from the Fraser River drainage. Four different simulations were performed, differing in (a) baseline sample size; (b) whether population allele frequencies were identical at both loci ($P = Q = \hat{\bar{P}}$ from Table 3) or were allowed to differ ($\hat{P}$, $\hat{Q}$ from Table 3); and (c) whether baseline allele frequencies in each replicate were adjusted[a] if monomorphic. In all cases, the mixture contained 500 fish. Actual mixture proportions varied randomly among replicates (see text for discussion). Results shown are means over 1000 replicates for the "old" method for handling baseline allele frequencies [using a single average frequency ($\hat{\bar{P}}$) for both loci] and the "new" method [using the isolocus estimates ($\hat{P}$, $\hat{Q}$)].

	Simulation							
	4		5		6		7	
Population frequencies	$\hat{P}$, $\hat{Q}$		$\hat{P}$, $\hat{Q}$		$P = Q = \hat{\bar{P}}$		$P = Q = \hat{\bar{P}}$	
Baseline sample size	100		25		100		100	
Baseline frequencies adjusted[a]?	No		No		No		Yes	
	Old	New	Old	New	Old	New	Old	New
Error[b]	0.061	0.045	0.080	0.068	0.047	0.048	0.050	0.051
Iterations[c]	314	265	313	257	318	297	350	325
Log likelihood	−1047	−1042	−1062	−1055	−1048	−1048	−1093	−1094

[a]Monomorphic baseline frequencies (0 or 1.0) adjusted to [0 + 1/(4N)] and [1.0 − 1/(4N)], respectively.
[b]Mean difference per population between true and estimated mixture proportions.
[c]Number of iterations required for EM algorithm to converge.

River simulations differed from those discussed previously in that the true population allele frequencies are not known; rather, they were estimated from relatively small samples (40–80 fish per population). In particular, the degree to which P and Q differ at each isolocus is unknown. Accordingly, two types of mixtures were analyzed: those in which the true population frequencies were set to the ML estimates ($\hat{P}$, $\hat{Q}$) obtained from the samples shown in Table 3, and those in which the true frequencies were both set to $\hat{\bar{P}}$, as also shown in Table 3. Note that in the former case, some of the ML estimates were the same at both loci, but generally they differed.

Results of these simulations again demonstrate the general advantages of the new method. With true population frequencies set to the actual ML estimates (simulation 4), the new method led to a substantially smaller mean error than the old method (0.045 vs. 0.061), and performance was also better as judged by number of iterations and log-likelihood of the solution (Table 4). The same qualitative result was obtained if base-

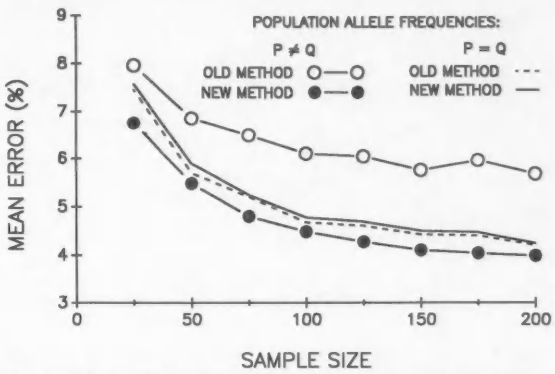

FIG. 1. Effects of baseline sample size on mean error in estimating true mixture proportions for simulated mixtures of five Fraser River chinook salmon populations. Lines with symbols are results for simulations using different population allele frequencies ($\hat{P}$, $\hat{Q}$ from Table 3) for each locus of an isolocus pair; lines without symbols are for simulations using the same allele frequency ($\bar{\hat{P}}$ from Table 3) for both loci.

line sample size was reduced to 25 (simulation 5), although the resulting increase in sampling error caused a general decline in the performance of both methods. Note also that with the smaller sample size, the difference between the old and new methods was less than with a baseline sample size of 100. As shown in Fig. 1, the advantages of the new method were substantial with large samples, but results for the two methods converged as sample size declined. In these simulations using Fraser River allele frequency data, precision of the stock composition estimates increased steadily with sample size until baseline sample size reached about 100–150 individuals, after which point the improvement was small.

As was the case for simulations involving hypothetical data, the two methods led to very similar results by all three criteria if true population frequencies were the same at both loci (simulation 6). This was true even for small baseline samples (Fig. 1).

Simulation 7 was identical to simulation 6 except that monomorphic baseline allele frequencies (0 for one allele, 1.0 for the other) were adjusted to $0 + 1/(4N)$ and $1.0 - 1/(4N)$, respectively, where N is the baseline sample size. This adjustment, similar to that proposed by Berkson (1953) in a slightly different context, is one means of addressing a problem common to all GSI analyses; i.e. an allele present in a population may fail to appear in a finite baseline sample, in which case any fish in the mixture carrying that allele will theoretically have probability 0 of coming from that population. The adjustment used allows for the possibility that an unsampled allele may actually occur in a baseline population; such an approach has been discussed but not, to our knowledge, implemented in any GSI analyses. The adjustment has an additional effect in the treatment of isolocus data: if no baseline frequencies are allowed to be exactly 0 or 1.0, all phenotypes have a non-zero probability of originating from every population. This is not true if monomorphic baseline frequencies are allowed (e.g. in a population with $\hat{P} = 0.8$, $\hat{Q} = 1.0$, phenotype III and IV individuals are theoretically impossible). Because ML estimates with $\hat{Q} = 1.0$ may in some circumstances occur fairly frequently even if parametric Q is less than 1.0 (see Waples 1988), it seems important to consider a correction for this effect.

An exact comparison of results with and without this adjustment was accomplished by using the same random number seed (and hence creating the exact same mixtures) in simulations 6 and 7.

Results shown in Table 4 suggest that this adjustment may not be desirable. By all three criteria, and by either the old or new method, the unadjusted allele frequencies (simulation 6) performed better than those that had been adjusted (simulation 7). A similar result was found in other simulations for which this adjustment was used: mean error was always slightly larger for both methods when the adjustment was applied (R. S. Waples, unpubl. data).

Discussion

Two major points emerge from the results of the simulated mixtures:

1) Use of the new method of treating isolocus data increases precision in estimating contributions of source populations if true population frequencies differ at the two loci of an isolocus pair;
2) the new and old methods yield similar results if true population frequencies are the same at both loci.

Clearly, maximum benefits to using the ML estimates rather than $\bar{\hat{P}}$ are obtained if the true frequencies (P, Q) at an isolocus pair differ substantially. How often this occurs in natural populations is not yet clear. To the extent that alleles are exchanged between loci (= "residual tetrasomy"; May et al. 1982), frequencies at the two loci would tend to converge, in which case it makes little difference whether ($\hat{P}$, $\hat{Q}$) or $\bar{\hat{P}}$ is used. However, studies that have demonstrated independent assortment of some isoloci in salmonids (Gall and Bentley 1981; Allendorf and Thorgaard 1984) suggest that frequencies can be expected to differ in at least some cases. Data to support this hypothesis have recently been reported for two isolocus systems in Pacific salmon. The *GPI-1,2* isolocus in pink salmon (*O. gorbuscha*) and the *IDH-3,4* isolocus in chinook salmon can, through differential tissue expression, be resolved into two individual loci, and in both systems each of the variant alleles are largely or entirely restricted to just one of the loci (Shaklee et al. 1990).

Fortunately, it is not necessary to determine the true population frequencies at an isolocus system before choosing which method to use in mixed-stock fishery analysis. Because the ML estimates increase precision if $P \neq Q$ while performing nearly as well as the old method if $P = Q$, use of $\hat{P}$ and $\hat{Q}$ for isolocus data can be recommended as a general practice in GSI analyses. Although this paper has focussed on precision, we expect that the increased resolving power provided by the new method should also help to reduce bias of stock composition estimates. As explained in Methods, modifying existing GSI algorithms to incorporate the new method simply involves adjusting the routine which calculates the probabilities that a particular phenotype observed in the mixture originated in each of the potential source population.

It should also be noted here that two of the potential limitations of the ML estimates reported by Waples (1988) do not adversely affect their use for GSI purposes. The first limitation is that, for a given sample size, the error in estimating the true population allele frequencies (P, Q) at an isolocus system is greater than the error in estimating the single allele frequency at an unduplicated gene locus. This suggests that precision of

stock-composition estimates using isolocus data will never be as great as could be achieved by analyzing the two loci separately (as Shaklee et al. (1990) show may be possible for some systems). Regardless of the baseline sample size used, the ML estimates should provide precision equal to or greater than that provided by the old method (Fig. 1; Table 4). However, to take maximum advantage of the increased precision that the new method affords when $P \neq Q$, it may be necessary to take baseline samples larger than the 40 or so individuals that Wood et al (1987; p. 873) found was a critical sample size "above which errors remain relatively constant." Figure 1 suggests that for isolocus data, the critical baseline sample size is closer to 100 individuals than 40. To a certain extent, however, the effects of sample size on estimates of mixture proportions depend on genetic differences among contributing stocks, and it is therefore not possible to provide a single, absolute guideline.

The second potential limitation of the ML estimates pointed out by Waples (1988) is that, because of the inherent symmetry of the isolocus problem, in a given sample it is not possible to determine whether $\hat{P}$ estimates the frequency at L_1 and $\hat{Q}$ the frequency at L_2, or vice versa. Therefore, one should not compare individual ML estimates (say, for $\hat{P}$) among populations; the ML estimates must be considered as a pair. In one sense this is unfortunate, because power to resolve the mixture would be enhanced if we could treat L_1 and L_2 separately. Again, however, the ambiguity between L_1 and L_2 does not present a problem for the proposed use of the ML estimates in GSI analyses. Only the joint phenotypic distribution (for both L_1 and L_2) is used in computing likelihoods, and expected phenotypic frequencies are identical whether computed using $\hat{P}$ at L_1 and $\hat{Q}$ at L_2, or the reverse.

The results of simulation 7 suggest that any benefits to be gained by adjusting monomorphic baseline frequencies as done here are outweighed by a loss in precision. However, the problem that this adjustment was designed to correct remains a real one. In any application of GSI, there is a high probability that the baseline samples will fail to detect all alleles present in the potential source populations. This is a general problem that pertains to unduplicated loci as well as isoloci, and further research may identify other means of dealing with it that increase precision of stock composition estimates.

Acknowledgements

We thank the Canadian Department of Fisheries and Oceans for providing the baseline samples of chinook salmon. Cindy Shiflett assisted in the collection and interpretation of the electrophoretic data. The efforts of Boyd Bentley (University of California, Davis) in developing screening protocols and a scoring model for *PGM-3,4* were essential to our ability to collect data for this isolocus system.

References

AEBERSOLD, P. B., G. A. WINANS, D. J. TEEL, G. B. MILNER, AND F. M. UTTER. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. NOAA Tech. Rp. 61: 1–19.

ALLENDORF, F. W., AND G. H. THORGAARD. 1984. Tetraploidy and the evolution of salmonid fishes, p. 1–53. *In* B. Turner [ed.] Evolutionary genetics of fishes. Plenum Press, New York, NY.

ALLENDORF, F. W., F. M. UTTER, AND B. P. MAY. 1975. Gene duplication within the family Salmonidae: II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations, p. 415–432. *In* C. L. Markert [ed.] Isozymes IV. Genetics and Evolution. Academic Press, New York, NY.

BEACHAM, T. D., A. P. GOULD, R. E. WITHLER, C. B. MURRAY, AND L. W. BARNER. 1987. Biochemical genetic survey and stock identification of chum salmon (*Oncorhynchus keta*) in British Columbia. Can. J. Fish. Aquat. Sci. 44: 1702–1713.

BERKSON, J. 1953. A statistically precise and relatively simple method of estimating the bio-assay with quantal response, based on the logistic function. J. Am. Statist. Ass. 48: 565–599.

COOPER, D. W. 1968. The significance level in multiple tests made simultaneously. Heredity 23: 614–617.

GALL, G. A. E., AND B. BENTLEY. 1981. Para-albumin polymorphism: an unlinked two-locus system in rainbow trout. J. Hered. 72: 22–26.

MARSDEN, J. E., C. C. KRUEGER, AND B. MAY. 1989. Identification of parental origins of naturally produced lake trout in Lake Ontario: application of mixed-stock analysis to a second generation. N. Am. J. Fish. Manage. 9: 257–268.

MAY, B., J. E. WRIGHT, AND K. R. JOHNSON. 1982. Joint segregation of biochemical loci in Salmonidae. III. Linkage associations in Salmonidae including data from rainbow trout (*Salmo gairdnerii*). Biochem. Genet. 20: 29–40.

MILNER, G. B., D J. TEEL, F. M. UTTER, AND C. L. BURLEY. 1981. Columbia River stock identification study: validation of method. Ann. Rep. Res. NOAA, National Marine Fisheries Service, Northwest and Alaska Fisheries Center, Seattle, Washington. 35 p.

PELLA, J. J., AND G. B. MILNER. 1987. Use of genetic marks in stock composition analysis, p. 247–276. *In* N. Ryman and F. Utter [ed.] Population genetics and fishery management. University of Washington Press, Seattle, WA.

SHAKLEE, J. B., C. BUSACK, A. MARSHALL, M. MILLER, AND S. R. PHELPS. 1990. The electrophoretic analysis of mixed-stock fisheries of Pacific salmon, p. 235–265. *In* Z–I. Ogita and C. L. Markert [ed.] Isozymes: structure, function, and use in biology and medicine. Wiley-Liss, Inc., New York, NY.

UTTER, F., P. AEBERSOLD, AND G. WINANS. 1987a. Interpreting genetic variation detected by electrophoresis, p. 21–45. *In* N. Ryman and F. Utter [ed.] Population Genetics and Fishery Management. University of Washington Press, Seattle, WA.

UTTER, F. M., D. J. TEEL, G. B. MILNER, AND D. MCISSAC. 1987b. Genetic estimates of stock compositions of 1983 chinook salmon, *Oncorhynchus tshawytscha*, harvests off the Washington coast and Columbia River. U.S. Fish. Bull. 85: 13–23.

WASHINGTON DEPARTMENT OF FISHERIES. 1988. Analysis of the 1987 Washington May troll chinook fishery. GSI Summary Rep. 88-2. Olympia, Washington.

WAPLES, R. S. 1988. Estimation of allele frequencies at isoloci. Genetics 118: 371–384.

WOOD, C. C., S. MCKINNELL, T. J. MULLIGAN, AND D. A. FOURNIER. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. 44: 866–881.

Influence of Exchange Flow Between the Channel and Hyporheic Zone on Nitrate Production in a Small Mountain Stream

Frank J. Triska, John H. Duff, and Ronald J. Avanzino

U.S. Geological Survey, Water Resources Division, 345 Middlefield Road, Menlo Park, CA 94025 USA

Triska, F. J., J. H. Duff, and R. J. Avanzino. 1990. Influence of exchange flow between the channel and hyporheic zone on nitrate production in a small mountain stream. Can. J. Fish. Aquat. Sci. 47: 2099–2111.

Variation in local exchange of flows between the channel and hyporheic zone produced temporally shifting concentration gradients of dissolved oxygen, nitrate, and ammonium in subsurface waters of a small, gravel-cobble bed stream. Channel water advected laterally supplied dissolved oxygen, and groundwater supplied ammonium to support hyporheic nitrification. Nitrate production was highest in sediment slurries from aerobic hyporheic sites, was absent at nearly anoxic sites, and was stopped by nitrification inhibitors (chlorate and nitrapyrin). Ammonium amendment to sediment slurries only slightly enhanced nitrate production indicating that sorption competed with biota for available substrate. Nitrate concentration increased from 75–130 μg N/L during 9 d of ammonium amendment to a hyporheic subsurface flow. Ammonium concentration rose slowly relative to a sulfate tracer initially, and declined slowly after cutoff as ammonium desorbed. Nitrate levels remained elevated for 6 d after cutoff as desorbed ammonium became biotically available. Interactions between the channel's hydrology, lithology, and biology such as we observed in nitrate production are probably more common than reported. However, the magnitude of the resulting nutrient flux will depend on factors which determine the depth and lateral extension of suitable hyporheic habitat.

La fluctuation observée dans l'échange local de débits canal-zone hyporhéique est à l'origine de gradients de concentration qui changent temporairement pour l'oxygène dissous, le nitrate et l'ammonium des eaux superficielles d'un petit cours d'eau au lit de galets et de gravier. L'eau du canal, sous l'effet d'une advection latérale, a fourni de l'oxygène dissous et les eaux souterraines, de l'ammonium pour la nitrification hyporhéique. La production de nitrate était la plus forte dans les boues sédimentaires provenant des endroits hyporhéiques aérobiques, elle était nulle dans les endroits presque anoxiques et elle était stoppée par les inhibiteurs de nitrification (chlorate et nitrapyrine). L'enrichissement en ammonium des boues sédimentaires n'a amélioré que légèrement la production de nitrate, ce qui indique que les processus de sorption ont concurrencé le biote pour le substrat disponible. La concentration de nitrate a augmenté, passant de 75 à 130 μg N/L pendant 9 j d'enrichissement en ammonium d'un écoulement souterrain hyporhéique. Au départ, la concentration d'ammonium a augmenté lentement par rapport à un traceur au sulfate pour ensuite baisser lentement après épuisement, à mesure que l'ammonium était désorbé. Les teneurs en nitrate sont restées élevées pendant 6 j après l'épuisement, car l'ammonium désorbé devenait biologiquement accessible. Les interactions entre l'hydrologie, la lithologie et la biologie du canal telles qu'elles ont été observées dans la production du nitrate sont probablement plus courantes qu'on ne le signale. Cependant, l'importance du flux d'éléments nutritifs qui en résulte dépendra de facteurs qui déterminent la profondeur et l'étendue latérale de l'habitat hyporhéique convenable.

Received July 19, 1989
Accepted May 15, 1990
(JA234)

Reçu le 19 juillet 1989
Accepté le 15 mai 1990

Biotic processes in streams, more than any other ecosystem, depend on a continual input of allochthonous resources in conjunction with internal recycling to maintain ecosystem function. Above ground vectors such as wind and gravity typically introduce large quantities of particulate organic matter while dissolved components penetrate the channel's subsurface in groundwater. Although considerable effort has been expended to estimate the source, quality, and process mechanisms for particulate resources, little effort has been expended on dissolved subsurface constituents (Hynes 1983; Likens 1984; LaBaugh 1986). Recent exceptions include reports of nitrate loss in subsurface flows beneath the riparian zone adjacent to cropped agricultural fields (Peterjohn and Correll 1984; Lowrance et al. 1984, 1985), and in the hyporheic zone of a Sonoran desert stream (Grimm and Fisher 1984). Triska et al. (1989b) also found hyporheic areas may act as a source or a sink for nitrogen under conditions of nitrate enrichment. Comparing groundwater and surface water chemistry, Stanford and Ward (1988) and Ford and Naiman (1989) found higher dissolved inorganic nitrogen concentrations in groundwater. Stanford and Ward (1988), Ford and Naiman (1989), and Triska et al. (1989b) hypothesized that ammonium supplied in groundwater is oxidized to nitrate during passage through the hyporheic zone. However, none of the above studies included direct measurement of processes responsible for hyporheic nitrate flux.

Interpreting data from hyporheic studies is difficult due to the problem of defining the groundwater-stream water boundary. In rivers with coarse gravels the lateral hyporheic zone can extend out to 3 km from the wetted channel (Standard and Ward 1988) providing extensive interstitial volume for invertebrate habitat and nutrient cycling. In channels with fine grain sediments oxygenated hyporheic habitats are confined to a far shallower zone typically <40 cm (Cummins 1975; Strommer and

Smock 1989) to 90 cm vertically in gravel-sand mixes (Williams 1989), and 1–10 m inland from the bank (Bretschko 1981; Williams 1989). Chemical indicators of the groundwater–stream water interface have included isopleths of conductivity (Stanford and Ward 1988), and concentration breaks in nitrate and sulphide (Williams 1989). In our previous work, Triska et al. (1989b) operationally defined the stream to include subsurface locations consisting of >10% surface water. Based on our operational definition, K. E. Bencala (unpubl. data) used a multiple injection of conservative solutes to determine general flow directions from the stream to inland areas and along subsurface flows adjacent to the wetted channel. In conjunction with this work we simultaneously: (1) examined chemical gradients in subsurface water across the riparian zone and determined temporal variation in nutrient concentration during summer low flow, (2) estimated nitrification potential of channel and inland sediments using laboratory slurry studies, and (3) verified the nitrification potential with an ammonium enrichment to an inland subsurface flow. These aspects of our hyporheic investigations are the subject of this report.

Study Area

The study was conducted in a 220 m reach of Little Lost Man Creek, a third-order pool-and-riffle stream in northwestern California (USA). The watershed is long, narrow and faces northwest. The watershed area is 9.4 km^2 and ranges in altitude from 24–695 m (387 m mean altitude). Soils on the watershed, derived from the Franciscan complex of Mesozoic age, are unstable and numerous dormant slides have been reported along the banks (Iwatsubo et al. 1975).

Approximately 92% of the vegetation is old-growth coastal redwood forest (*Sequoia sempervirens*) including associated Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*). During the mid-1960's and prior to incorporation into Redwood National Park, 8% of the watershed was clearcut. Riparian vegetation is dominated by red alder (*Alnus rubra*), a nitrogen fixing species.

The overall channel gradient is 0.066 (Iwatsubo and Averett 1981) and 0.018 in the study reach. Channel sediments are large and poorly sorted and particulate organic matter commonly ranges in size from fine particulates to large logs. The bed is gravel-cobble and is highly permeable with significant interflow of discharge. Summer stream water temperature varies between 14°C and 20°C. The periphyton community is dominated by diatoms, and a full range of invertebrate functional groups are represented in the benthos (Iwatsubo and Averett 1981).

The area has cool wet winters and warm dry summers. Average annual precipitation is 178 cm at a rain gage approximately 3 km northwest of the study site. Definite wet and dry seasons, and the high permeability of streambed sediments make Little Lost Man Creek an excellent natural laboratory for studies of hyporheic nitrogen cycling. Monthly precipitation historically peaks in January at 32.0 cm, and is typically >12.5 cm/month October through April. Mean summer precipitation is low (2.5 cm, 0.6 cm, and 2.1 cm, June–August respectively). As a result, physical disturbance of the bed and its associated epilithic flora is minimal May–October. Anomalies between the historic pattern and the years of our study included low precipitation in January of both years, and high precipitation in November, 1984. The study period in 1984 had slightly higher than normal precipitation in early spring, whereas 1985 was slightly lower. Within the study period of both years mean monthly precipitation declined throughout summer with July and August being the driest months. Both summers would be considered historically typical.

Materials and Methods

Well Installation

In October, 1983, four transects of wells were installed along a 220 m reach of Little Lost Man Creek. The wells were sunk by hand to penetrate the water table (40–60 cm, summer base flow conditions) and were then backfilled against a 15 cm polyvinyl chloride pipe and the top was capped (Fig. 1). The base remained open and sides of the casing were crudely screened by cutting large slits through the bottom 20 cm of pipe permitting nearly unrestricted flow. Above the water table a styrofoam plug wrapped in aluminium foil minimized direct gaseous exchange with the atmosphere. Water was sampled through a stainless steel "J" tube submerged in the well (Fig. 1). Transect A, referred to as the alder wells, was located at the base of a forested slope clearcut in 1965 (Fig. 2). Riparian vegetation was alder, which also constituted a significant component of upslope vegetation. The wells (WA1–WA4) were located 0.9–13.2 m perpendicular to the wetted channel. At winter base flow WA1 was within the wetted channel, while in summer it was along the margin. Wells B1, C1, and D1, were at the bank of the incised channel. Other wells were inland of the bankfull channel at all times. A small tributary (Rivulet) and its well (WB2) were sampled to compare subsurface and surface tributary chemistry near a groundwater source. Combined surface and subsurface flow of the tributary was <1.0% of channel discharge. Thus the tributary was an insignificant input of water and nutrients. Transect C was located across a large gravel deposit colonized by alder. The gravel bar wells (WC1–WC3) were located 4.0–12.0 m perpendicular to the wetted channel. WC4 was approximately 6.0 m inland from the riparian zone and 18.0 m from the wetted channel. Vegetation at this site was sword fern (*Polystichum munitum*), a common understory species. The sediments were soil, with minimal gravel, although this well was located at the base of an old alluvial deposit. Transect D was at the base of a steep slope dominated by undisturbed old-growth redwood, 0.6–11.4 m from the wetted channel. Only well WD1 was in an area of unconsolidated gravel. The inland wells (WD2, WD3) were in forest soils with a minimal gravel component. Following installation, the wells were left undisturbed over winter.

Water Sampling and Analysis

Well and stream sites were sampled May 22, July 20, August 3, and one to four times daily August 15–30, 1984. Samples for dissolved inorganic constituents were pumped peristaltically through silicone rubber tubing with an inline filter containing a 0.45 μm Millipore[1] HATF (no surfactant) membrane to new polyethylene bottles prerinsed three times with sample. Sulfate was analyzed by ion chromatography (Dionex 2000i). Nitrate plus nitrite-nitrogen, henceforth referred to as nitrate, was determined by an automated cadmium reduction method (Technicon Industrial Method #158-71W, December, 1972)

[1]Use of brand names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

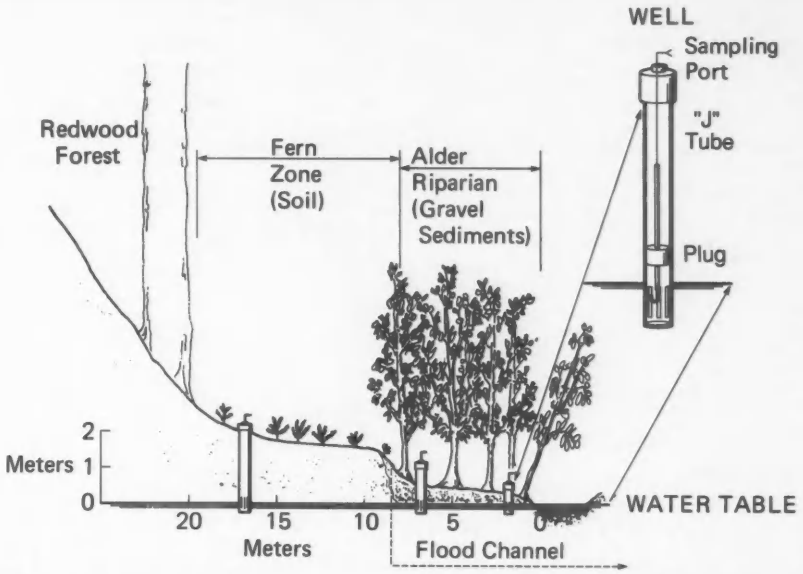

FIG. 1. Schematic diagram of a typical well and well transect, showing its relationship to the channel, water table, and sediment and floristic zones.

for field samples, or by ion chromatography for slurry studies. Ammonium was determined using an automated phenol-hypochlorite method (Technicon Industrial Method #154-71W, February 1973). All dissolved inorganic nitrogen (DIN) concentrations are reported as μg N/L. Dissolved oxygen (DO) was measured with a YSI 57 oxygen meter.

Samples for dissolved organic nitrogen (DON) were pumped through an oven-baked, in-line, glass fiber filter, directly into oven-baked glass serum bottles and later refiltered through silver membrane filters. Twenty millilitre subsamples were subsequently placed in quartz tubes and oxidized by hydrogen peroxide and ultraviolet radiation to convert total dissolved nitrogen (TDN) to nitrate. Dissolved organic nitrogen was obtained as the difference between TDN and DIN (Armstrong, et al. 1966; Armstrong and Tibbitts 1968; Manny et al. 1971).

Sediment Slurry Experiments

Nitrification was assayed in the stream and subsurface habitats in a series of experiments using sediments slurried in stream water, and shaken at room temperature (18–20°C). Sediments were collected from the wells using a plastic scoop at the end of a 1.8 m pipe. The nitrification potential of sediments from an oxic site (WA2) with high nitrate and low ammonium concentration was contrasted with sediments from a nearly anoxic site (WC4) with high ammonium and low nitrate. Heterotrophic nitrification (nitrate produced from the oxidation of organic nitrogen) vs. autotrophic nitrification (oxidation of ammonium) was also compared. Sediment was slurried (20 mL sediment to 100 mL stream water) in 250 mL flasks. All flasks were amended with 0.5 mM NH_4 and 0.1 mM PO_4 (final concentration) to avoid potential nutrient limitation. Triplicate flasks of non-inhibited slurries and five nitrification inhibited controls included: (1) boiling, (2) nitrapyrin (2-chloro-6-trichloromethyl-pyridine), an inhibitor of ammonium oxidation (10 μL of 22% solution/flask), (3) sodium chlorate ($NaClO_3$), an inhibitor of nitrite oxidation (20 mM final concentration), (4) acetylene (C_2H_2), an inhibitor of ammonium oxidation (15 mL into the headspace), and (5) acetylene plus tryptone (100 mg/L final concentration), an organic nitrogen source for heterotrophic oxidation. Acetylene amended flasks were sealed with rubber stoppers, others were cotton stoppered then shaken 5 min when an initial 20 mL water sample was withdrawn, filtered, and analyzed for nitrate. Additional samples were taken after 5 h.

Nitrification potential was next assayed using sediments from Transect A (WA1-4). All slurries (20 mL sediment to 100 mL stream water in 250 mL flasks) were ammonium and phosphate amended as before. Water (10 mL) was sampled after 5 min and after 24 and 48 h.

Nitrate production was estimated as a function of ammonium concentration. Sediment from WA2 was slurried in duplicate with and without amonium to achieve the following final concentrations: background (no amendment), 5 μM, 10 μM, 25 μM, 100 μM, 500 μM, 1000 μM. Samples were taken after 5 min and 2, 4, 8, and 20 h for ammonium amendments <50 μM. Concentrations >50 μM were sampled immediately and after 8 and 20 h. Nitrapyrin treated bottles (10 μm, 100 μm, and 1000 μm ammonium) were sampled immediately and after 20 h only.

On October 4, 1985, sediments were removed from the stream channel and wells WA1-WA4, Rivulet, WB1-WB2, WC1, WC3, and WD1-WD3. Sediments slurries (25 mL of fresh sediment to 100 mL of stream water) were treated as before (background unamended, nutrient amended, nutrient amended plus nitrapyrin). The flasks were sampled (10 mL) after 5 min and after 16 and 40 h.

Field Experiment for Nitrification Potential

To determine the potential for nitrification under natural hydrologic conditions ammonium ($(NH_4)_2SO_4$) was coinjectioned with sulfate ($MgSO_4$) to a pit approximately 3 m upflow

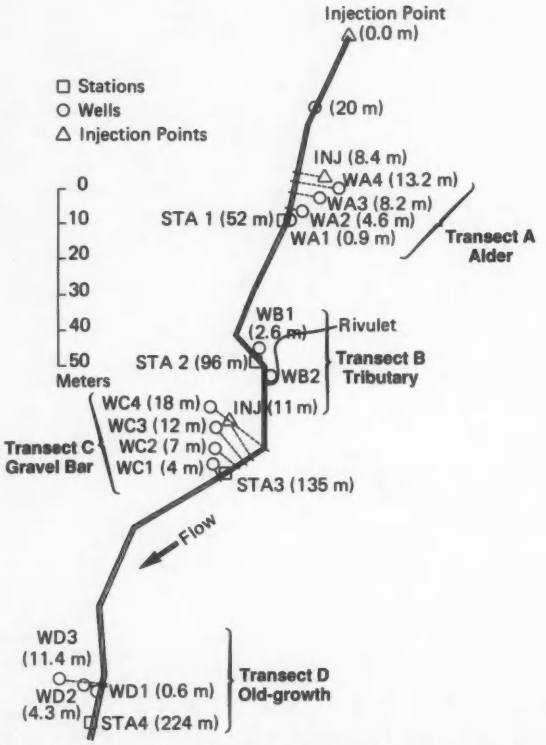

FIG. 2. Map of the experimental reach illustrating stream sampling stations (□), well locations (○), and injection locations (△). For station locations the number in parenthesis is the distance from the head of the reach, for wells and injections sites it is the distance perpendicular to the channel along a line of sight indicated by the dashed line.

from WC3 (Fig. 2). Sulfate was selected as the hydrologic tracer since the flow was highly aerobic, which minimized the possibility of sulfate reduction. Sulfate was also used because chloride and bromide were being simultaneously used at other locations (K. E. Bencala, pers. comm.). The ammonium injection began at 1400 August 15, and nutrients were cut off at 1400 August 23. Samples were taken approximately four times daily between August 15–23, and approximately once daily until August 30. Water was analyzed for ammonium and nitrate by automated wet chemistry, and sulfate by ion chromatography.

Results

Chemical Gradients

Dissolved oxygen

Dissolved oxygen decreased temporally in channel water and decreased both temporally and laterally away from the stream channel in subsurface waters, May through August (Table 1). Lower temperature and higher discharge resulted in higher DO in the stream channel in early spring (12.2 mg/L) than late summer (9.5 mg/L). Along Transect A, DO decreased laterally from 12.2 (WA1) to 8.8 mg/L 13.2 m inland in May (Table 1). By August 2, DO was reduced from 8.8 to 1.5 mg/L in the most inland well. While the small surface tributary (Rivulet) had a DO concentration similar to the channel in May, a subsurface water at the rivulet-stream junction (WB2) was oxygen-depleted on all dates, averaging 1.4 mg/L. Highly aerobic conditions prevailed at gravel bar sites WC1-WC3 throughout summer. However, subsurface water further inland (WC4) was oxygen-depleted, averaging only 1.4 mg/L. At the old-growth transect, water up to 4.3 m inland (WD1-WD2) was highly oxygenated, whereas WD3, 11.4 m inland, was significantly lower in DO than other wells and nearly oxygen-depleted by August.

TABLE 1. Oxygen concentration (mg/L) in the stream channel and in subsurface flows adjacent to Little Lost Man Creek during May-August, 1984.

Site number	Distance from channel (m)	May 24	July 19	August 2
		Transect A (alder)		
Stream	0.0	12.2	9.8	9.5
WA1	0.9	12.2	9.8	8.9
WA2	4.6	10.0	4.8	3.3
WA3	8.2	9.8	3.0	3.0
WA4	13.2	8.8	2.3	1.5
		Transect B (tributary)		
Stream	0.0	12.2	9.8	9.5
WB1	2.6	—	6.8	6.3
WB2	1.0	2.2	0.5	1.5
Rivulet	—	11.0	—	—
		Transect C (gravel bar)		
Stream	0.0	12.2	9.8	9.5
WC1	4.0	11.6	8.6	7.5
WC2	7.0	9.8	7.9	7.3
WC3	12.0	9.8	6.0	5.1
WC4	18.0	0.8	1.0	2.5
		Transect D (old-growth)		
Stream	0.0	12.2	9.8	9.5
WD1	0.6	10.8	7.6	8.0
WD2	4.3	9.8	8.1	7.2
WD3	11.4	3.2	1.4	1.0

Nitrate

Channel nitrate concentration exhibited seasonal, diel, and upstream-downstream trends. Nitrate concentration of channel water increased between spring and late summer. On May 22, nitrate averaged 7.4 ± 1.2 SD μg/L. Concentration doubled by August 3 to 17.4 ± 2.0 μg/L, and doubled again by August 16 to 37.7 ± 5.5 μg/L (Fig. 3). Nitrate concentration between August 15–25 also decreased at midday and increased spatially between Stations 1 and 4 (Fig. 4). The lowest daily nitrate concentration was typically at 1400, the highest before dawn (0600). Daytime decreases of 25–40% of night concentration were attributed to uptake by photoautotrophs. While uptake patterns were similar, instantaneous nitrate concentration was always higher at Station 4. This indicated a nitrate source to the reach despite the high demand for epilithic production. Synoptic sampling of intermediate stations between Station 1 and Station 4 (data not shown) indicated a gradual downstream increase of nitrate concentration. Gradual change and absence of significant tributary input was evidence for sustained incremental nitrate accretion along the reach rather than a highly concentrated point source.

Nitrate in the well transects was typically higher than in the wetted channel (Fig. 3). In WA1, which was within the wetted

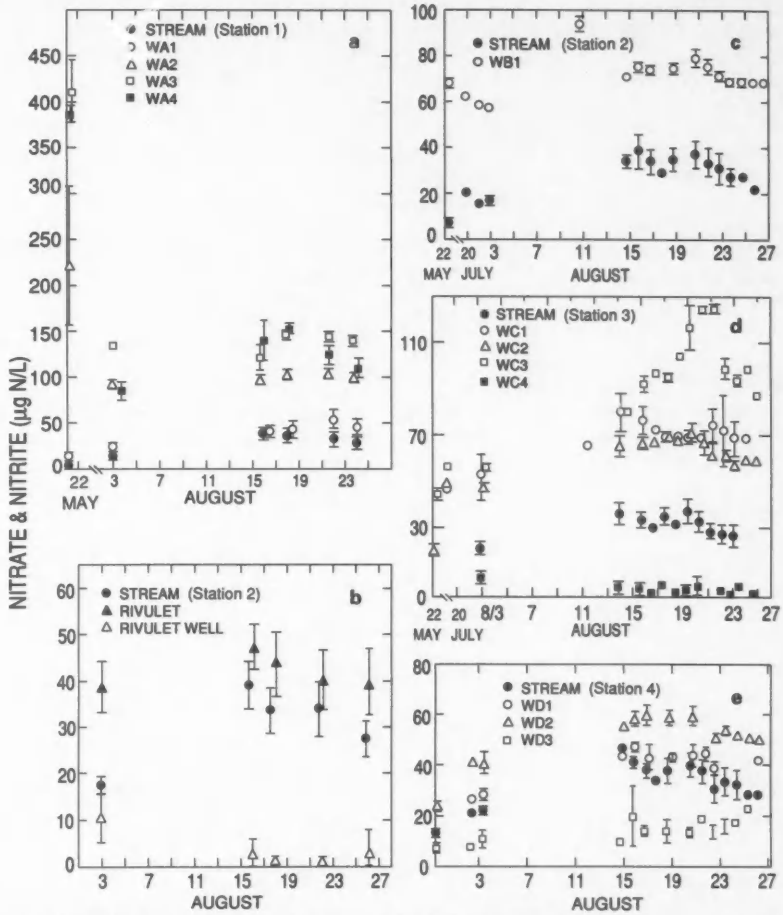

FIG. 3. Comparison of nitrate concentration: (1) temporally between spring and late summer, and (2) spatially along each well transect. Error bars are one standard deviation for dates when three or more samples were collected within a 24 h period. Wells are coded as in Fig. 2.

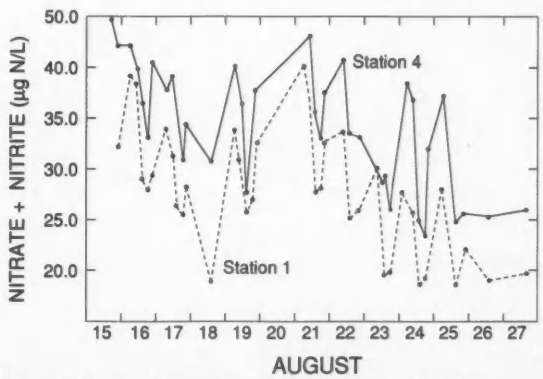

FIG. 4. Difference between upstream (Station 1 - - - -) and downstream (Station 4 ____nitrate concentration August 15–27, 1984.

channel most of the year, this difference was indistinguishable in May, but became more significant as the well increasingly separated from the wetted channel by seasonal dewatering. On May 22, the first well outside the bankfull channel (WA2) had an average nitrate concentration of 233.7 ± 76.5 µg/L compared to 7.4 ± 1.2 µg/L in the channel only 4.6 m away (Fig. 3a). Nitrate concentration was even higher at WA3 and WA4, averaging 410.0 ± 32.0 µg/L and 388.0 ± 7.0 µg/L, respectively. Nitrate concentration in the alder wells decreased in conjunction with the decrease in DO. By August 3, wells WA2 and WA4 had less than 100 µg/L and WA3 less than 150 µg/L. Throughout August, nitrate concentration remained significantly higher in the more inland wells WA2-4, than in the stream or WA1.

Nitrate was first sampled at the tributary (Rivulet) August 3. On that date nitrate concentration in the tributary was higher than in channel water (Fig. 3b). After August 15, when channel nitrate doubled, differences were not significant. Nitrate concentration in both stream and tributary water always exceeded the Rivulet well (WB2), which was typically <10 µg/L. At WB1, nitrate concentration consistently exceeded both adjacent

stream water and the rivulet well (Fig. 3c), indicating hydrologic separation from the tributary.

Beneath the gravel bar (WC1-WC3), nitrate concentration also was consistently higher than adjacent stream water on all dates (Fig. 3d) and increased May through August. The nearly anoxic well (WC4) had minimal nitrate (≈10μg/L). Subsurface flows in the vicinity of Transect D had a pattern of nitrate concentration similar to the gravel bar wells (Fig. 3e). In both, nitrate concentrations were <50 μg/L in spring and did not exceed 100 μg/L during the summer. At the most inland well, WD3, nitrate concentration was always significantly lower than stream water, and coincided with low DO.

Ammonium

Ammonium concentration was always low in channel water (5–10 μg/L) and lacked either a diel or seasonal pattern (Fig. 5). Spatially, ammonium concentration more than doubled going inland (WA3 and WA4, Fig. 5a). Rivulet also had low ammonium concentration in surface water, but subsurface concentration (WB2) was up to 30 times higher (Fig. 5b). The highest ammonium concentration in wells coincided with low dissolved oxygen and low nitrate concentration. The gravel bar and old-growth transects were consistently low in ammonium, except at the most inland sites (Fig. 5d, 5e). In the poorly oxygenated water of WC4 maximum ammonium concentration exceeded 1.0 mg/L. Similarly, in the least oxygenated well on the old-growth transect (WD3) ammonium was typically higher than other wells, but rarely exceeded 30 μg/L.

Dissolved organic nitrogen

Dissolved organic nitrogen was a significant component of nitrogen in the channel and at most wells, but the concentration was highly variable. At typical channel water concentrations, approximately half of the TDN in transport was DON. Wells along the alder transect had mean diel concentrations of DON ranging between 35 and 60 μg/L (Fig. 6a). The alder wells were the only sites where DON was a minor component of TDN due to high nitrate concentration. DON concentration was high in both the Rivulet and WB2 (Fig. 6b). Rivulet DON ranged from 90–140 μg/L, and was higher in August, whereas at WB2 concentration generally decreased over time. Concentrations of DON ranged between 30–45 μg/L in the adjacent stream, and 50–70 μg/L at WB1 (Fig. 6c).

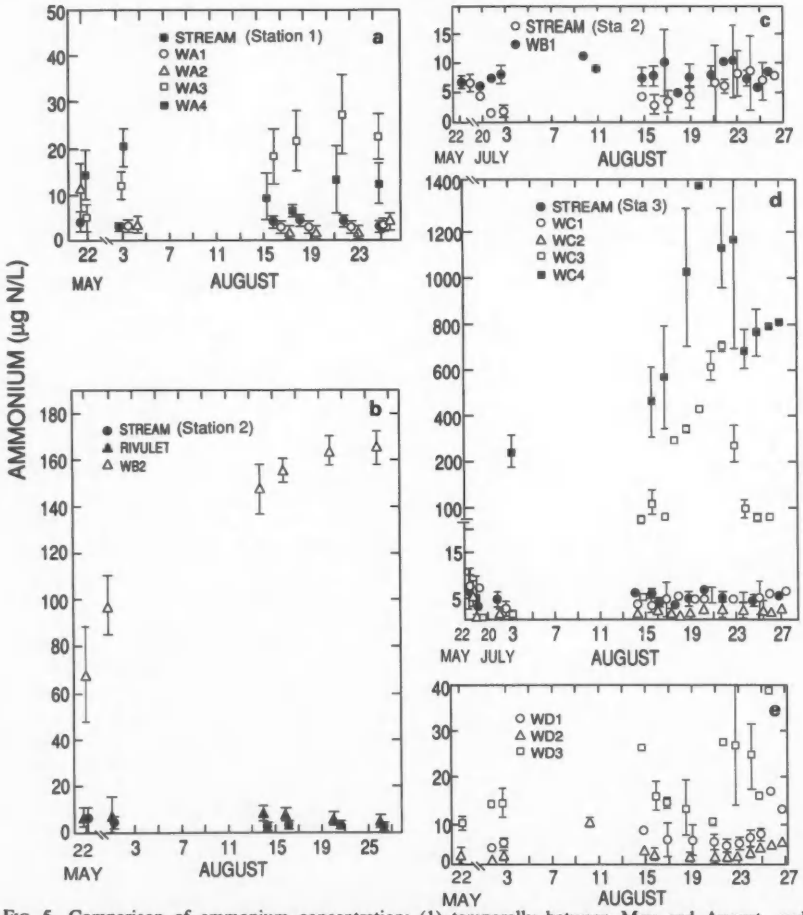

FIG. 5. Comparison of ammonium concentration: (1) temporally between May and August, and (2) spatially at all well transects. Error bars are one standard deviation when three or more samples were collected within a 24 h period. Wells are coded as in Fig. 2.

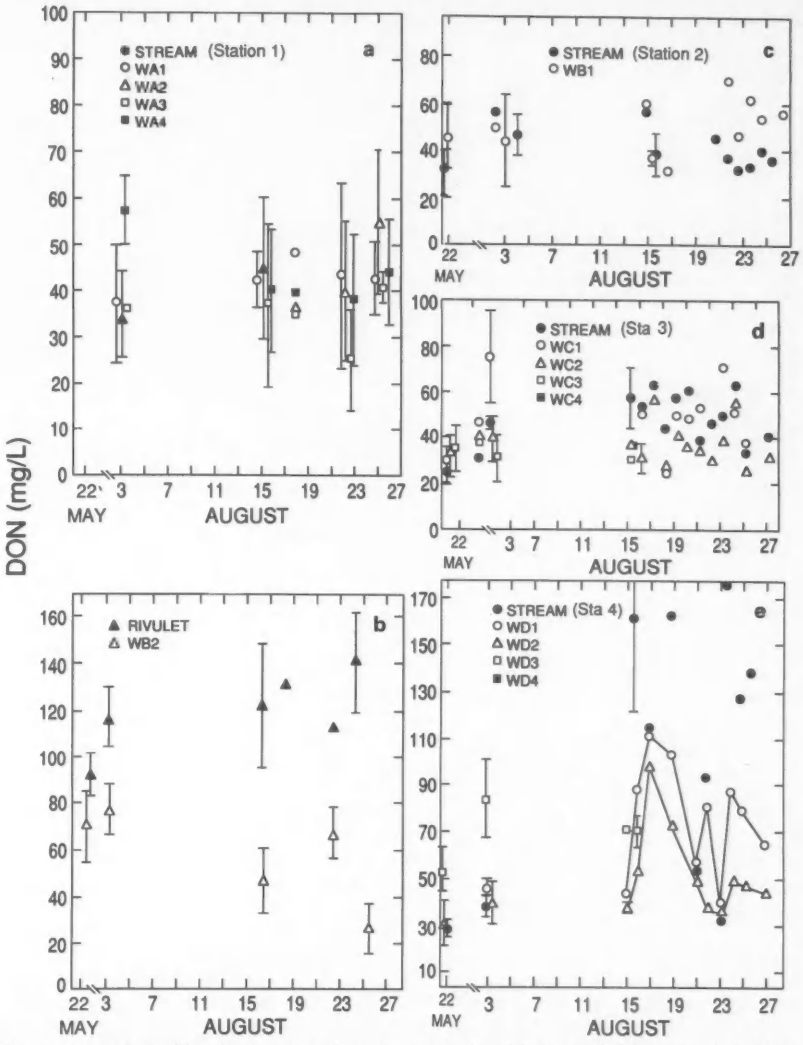

FIG. 6. Comparison of dissolved organic nitrogen concentration: (1) temporally between spring and late summer, and (2) spatially at all well transects. Error bars are one standard deviation when three or more samples were collected within a 24 h period. Wells are coded as in Fig. 2.

Under the gravel bar (WC1-WC3) DON concentrations ranged from 30–35 μg/L on May 22 (Fig. 6d). Concentration varied by location and date throughout summer, typically ranging between 25–60 μg/L. At the old-growth wells DON concentration was higher inland than at more bankside wells on May 22 and August 2–3 (Fig. 6e). A pulse of DON was observed August 15–17.

Sediment Slurry Experiments

Water chemistry from the channel and hyporheic water in the alder riparian wells hinted that nitrification was the source of the high nitrate levels in the hyporheic water adjacent to the channel in May. Incubation of sediment slurries from WA2 confirmed the presence of a nitrifying capacity in hyporheic sediments connected to the main channel by subsurface flows. During short-term assays, nitrate formation in sediment slurries from WA2 was stimulated by the addition of ammonium and inhibited by the addition of nitrapyrin and chlorate and by the boiling of sediments (Fig. 7a). Nitrapyrin effectively inhibited nitrate production in all slurry experiments. In contrast hyporheic sediments not closely connected to the main channel by subsurface flows lacked nitrification capacity (Fig. 7b). Ambient ammonium levels are typically elevated in hyporheic water not closely coupled to the stream, therefore the oxygen tension likely controls the nitrifying capacity of the sediments there. Hyporheic sediments amended with tryptone as an organic source for heterotrophic nitrification failed to stimulate nitrate formation at either WA2 or WC4 (Fig. 7c). While acetylene blocks ammonium oxidation in chemoautotrophic nitrification, it should not inhibit heterotrophic nitrification of DON (Schimel et al. 1984). Therefore, heterotrophic nitrification is

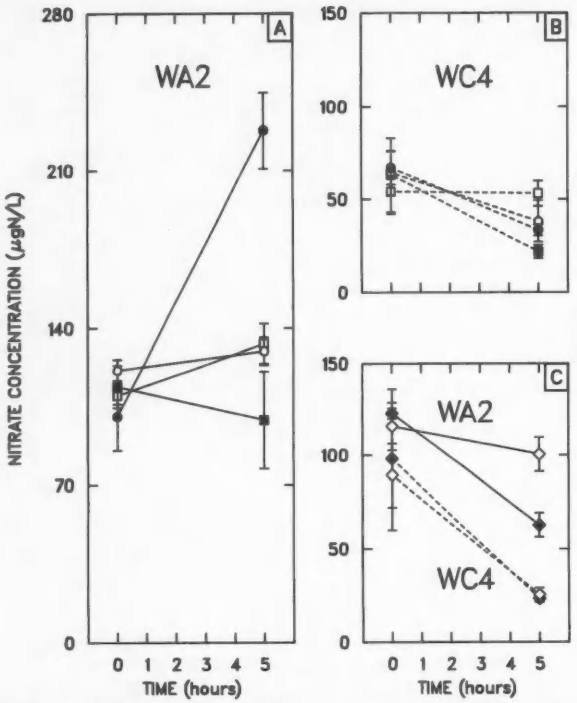

FIG. 7. Mean nitrate concentrations in ammonium-amended slurries from WC4, a nearly anoxic subsurface flow with high background ammonium concentration (- - -) and sediments from an oxic subsurface well (WA2) with low background ammonium concentration (___). The error bar is the standard deviation. Treatment symbols are as follows: (●) without inhibitor, (□) boiled, (○) chlorate, (■) nitrapyrin, (◇) acetylene, (◆) acetylene plus tryptone.

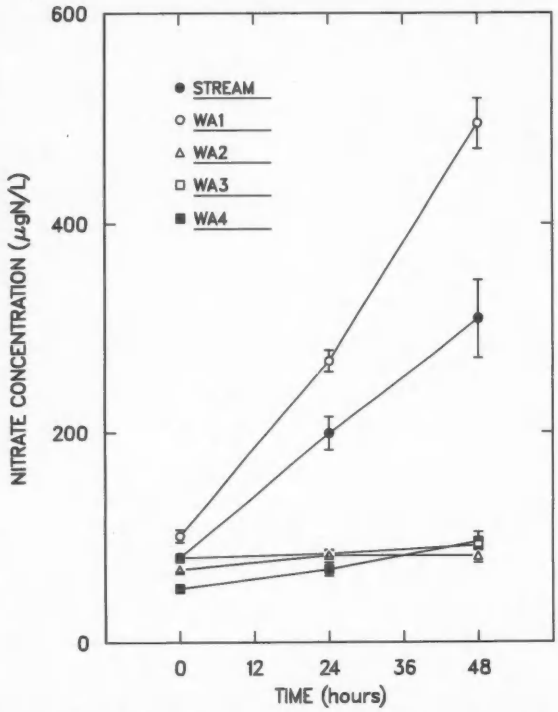

FIG. 8. Nitrate concentration in ammonium and phosphate amended sediment slurries collected from the stream and along Transect A at Little Lost Man Creek. Error bars are one standard deviation. Treatments were incubated in triplicate.

presumably an insignificant source of nitrate to hyporheic waters.

Nitrification potential generally decreased with increasing distance from the wetted channel. Nitrate concentrations increased three-fold within 48 h in channel sediments amended with ammonium (Fig. 8). Well WA1 had the highest nitrification potential, increasing more than five-fold. Sediments from mid-transect locations WA2 and WA3, 4.6 m and 8.2 m respectively from the channel, had little nitrate production, however, nitrate approximately doubled at WA4.

Nitrate increase was only weakly related to ammonium amendment, although some concentration effects were observed. Initial rates of nitrate formation were independent of initial ammonium concentrations (Fig. 9). By 8 h nitrate production slowed in the flasks at background concentration and with amendments <25 μM, whereas it continued in flasks with 50 and 100 μm ammonium amendments. The lowest amended slurries may have become ammonium-depleted by nitrate production and possibly sediment sorption. Slurries amended 50 and 100 μM above background had nearly linear rates of nitrate production over 20 h, possibly indicating enzyme saturation for the full experiment. Ammonium concentrations this high are rare in the field but have occasionally been observed in groundwater samples from WC4 and WB2. In the highest amendments, 500–1000 μM, concentration leveled either due to a slowing of nitrate production or increase in nitrate demand after 8 h. Decreased nitrate formation may have resulted from growth of other populations in the slurry community or onset of reducing conditions despite shaking. Final nitrate accumulation was similar to the 50 and 100 μM amendments.

Nitrification potential from the full spectrum of habitats was compared. The channel was at annual low flow October 3, 1985, so exchange flow through the hyporheic zone was minimal. The pattern of nitrification in the alder wells was similar to August 22 (Fig. 10). Differences included highest nitrification potential associated with channel sediments rather than WA1. The rate of nitrate production was similar for the first 16 h in both the ammonium amended and unamended slurries from the stream, WA1 and WA2 after which it slowed in the unamended slurries. In slurries from the most inland wells (WA3-WA4) nitrate formation was observed when ammonium was added, whereas nitrate loss was observed in unamended slurries.

Surface sediments of Rivulet had the highest nitrification potential of any bankside habitat (Fig. 11). As in the stream and WA1, nitrate increased rapidly in both unamended and ammonium-amended slurries by 16 h. Nitrate accumulation in the unamended slurry was subsequently depleted to approximately background concentration. Subsurface sediments of WB2 had little nitrate formation even with ammonium amendment. Slurries from the bankside well, WB1, had nitrate production rates just slightly lower than WA1 in amended slurries for the first 16 h. By 40 h the process either slowed or nitrate demand equalled production.

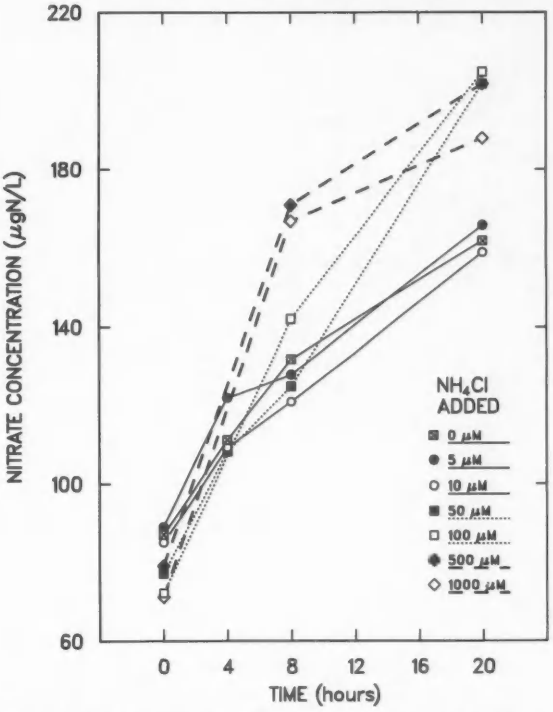

FIG. 9. Nitrate concentration in response to ammonium amendment in sediments from well WA2. Each point is the mean of duplicate treatments amended as indicated.

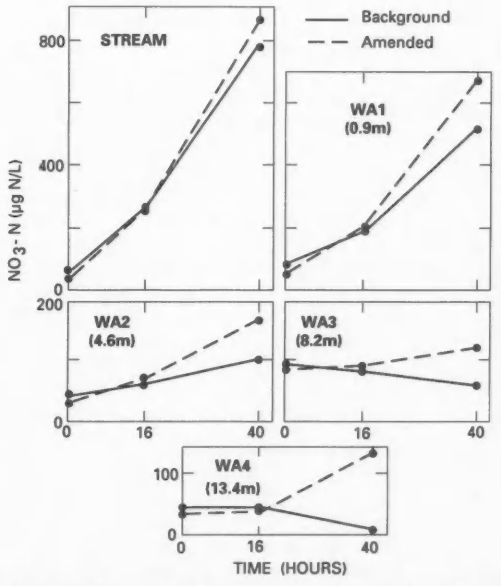

FIG. 10. Nitrate concentration during a 40 h incubation of sediments from the stream channel, and in subsurface sediments of Transect A. Slurries were incubated either at background conditions (____) or were ammonium amended (- - -).

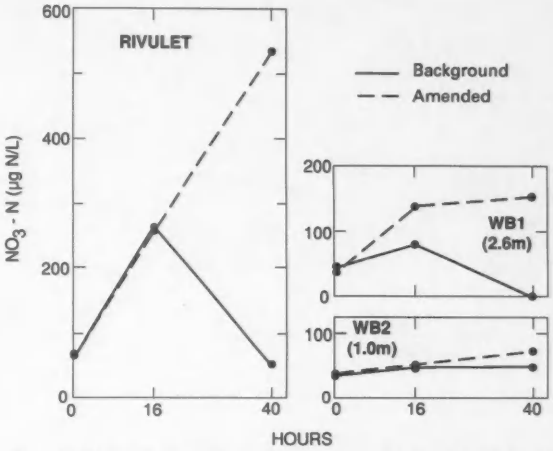

FIG. 11. Nitrate concentration during a 40 h incubation of sediments from Transect B. Slurries were incubated either at background conditions (____) or were ammonium amended (- - -).

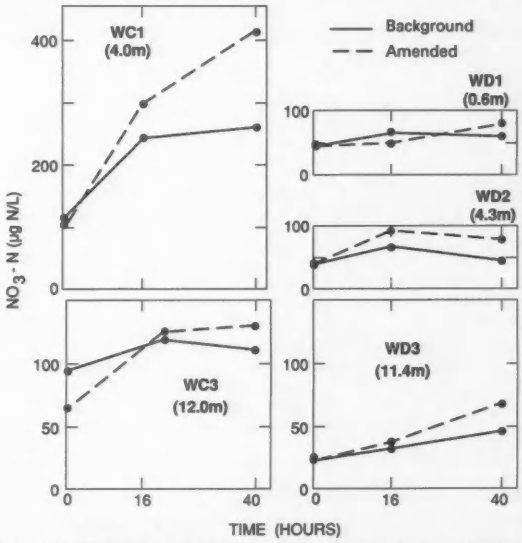

FIG. 12. Nitrate concentration during a 40 hour incubation of subsurface sediments along Transects C and D. Slurries were incubated either at background conditions (____) or were ammonium amended (- - -).

Sediments from the gravel bar well WC1 demonstrated nitrification potential under both background and ammonium-amended conditions (Fig. 12). Nitrate production was highest through hour 16. At WC1 nitrate production subsequently decreased without amendment, but only slightly with amendment. At WC3 there was little nitrate production after 16 h without ammonium amendment, however nitrate nearly doubled in the amended flask. Nitrification potential was absent in slurries from WC4.

At the old-growth transect (WD1-WD3), nitrification potential was lowest of the four study sites (Fig. 12). The amount of nitrate increase was slight at WD1 compared to other bankside locations (WA1, WB1, and WC1). Nitrification potential was also low at WD2 and WD3.

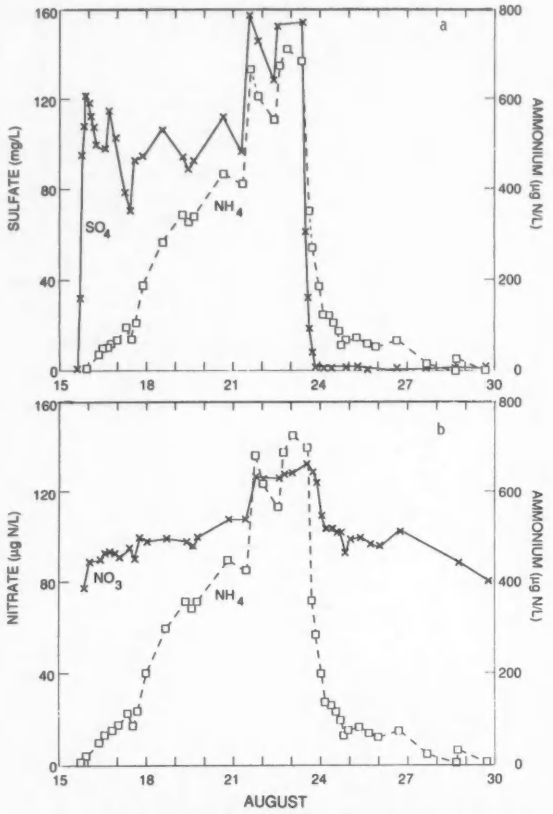

FIG. 13. (a) Concentration of sulfate (X) and ammonium (□) resulting from a solute coinjection to an injection well approximately 3 m upflow from sample well WC3 (see Fig. 2). (b) Concentration of nitrate (X) and ammonium (□) resulting from a solute coinjection to an injection well approximately three meters upflow from sample well WC3 (see Fig. 2). The injection began August 14, 1400, and ended August 23, 1400.

Field Experiment for Nitrification Potential

The slurry studies permitted a controlled evaluation of mechanisms regulating nitrate production in hyporheic sediments, and determined the spatial-temporal pattern. While control and replication were afforded by the slurry studies, they were artificial relative to in situ conditions. Temporal trends indicated that advection of dissolved oxygen regulated nitrate production during the dry season. Experimentally altering the volume of exchange flow in the field was not practical, so we attempted to confirm nitrate regeneration in situ by raising ammonium concentration. This field verification was analogous to the slurry studies and also tested the hypothesis that ammonium exchange between sediments and interstitial water regulated ammonium availability. The study site was WC3, an aerobic flow (Table 1), which had higher than channel nitrate (Fig. 3c), but where we could measure a relatively small increase in nitrate concentration. Concentration of the conservative tracer, sulfate, already began to rise when the first sample was taken after 3 h (Fig. 13a) indicating rapid throughput of water. Coinjected ammonium was not transported with sulfate, and within the first 2 d most ammonium was removed from the subsurface flow. Potential fates of coinjected ammonium included sediment sorption, biological oxidation to nitrate, and assimilatory uptake. The potential for sorption was indicated by the concentration pattern after "cutoff" on August 23. Sulfate decreased almost immediately to background concentration while ammonium attenuated over 6 d. Thus, sorption constituted a large proportion of ammonium retention. Concentration variations in sulfate along the plateau, August 16–24 (Fig. 13a) were due to variation in the groundwater vs. stream water composition.

Nitrate concentration increased about 12% within hours, in response to ammonium amendment (Fig. 13b). Nitrate concentration rose from 75 µg/L to a maximum of 130 µg/L at cutoff, 1000 August 23. In an adjacent well, WC2, which was minimally impacted by sulfate and not by ammonium, nitrate concentration remained at background. In WC3, sulfate decreased immediately, while nitrate persisted at near the maximum concentration >8 h after cutoff. Presumably, ammonium desorption provided sufficient substrate to retain nearly maximum nitrate production. As desorption continued for the next 6 d, nitrate concentration fell, but remained above background until August 30. The amount of assimilatory ammonium uptake is unknown.

The field results indicated the occurrence of nitrate production and provided preliminary confirmation of ammonium sorption to sediments. The increase in nitrate concentration was significant relative to background and indicated nitrification potential in natural subsurface flows. The concentration increase was especially significant since subsurface flow through WC3 was <3 h travel time. Desorption for 6 d following cutoff indicated sorbed ions were exchangeable with interstitial flows controlling ammonium availability under natural hydrodynamic conditions.

Discussion

Early studies of the hyporheic zone emphasized its importance as a habitat and as a refugia for aquatic invertebrates (e.g. Coleman and Hynes 1970; Hynes 1974; Williams and Hynes 1974; Hynes et al. 1976). More recent studies also indicate a site for nutrient flux both beneath the channel (Grimm and Fisher 1984) and laterally across the riparian zone (Peterjohn and Correll 1984; Lowrance et al. 1984, 1985; Stanford and Ward 1988; Ford and Naiman 1989; Triska et al. 1989b). The hyporheic zone, which can encompass a large range of depth and lateral distance from the wetted channel (cm to km), is a product of a landscape's physical properties (e.g. watershed area, gradient, grain size of sediment, and annual precipitation). Within this landscape perspective, temporal and spatial variability at the reach scale indicate the importance of identifying local hydrodynamic mechanisms which form, maintain and modify the location of the groundwater-stream water interface.

At Little Lost Man Creek the groundwater–stream water interface fluctutated with discharge producing a spatial/temporal pattern in chemical gradients as precipitation declined steadily from its winter peak. By April precipitation ($\bar{X}$ = 12.5 cm) was sufficient to keep soils saturated or nearly so. Therefore, most spring rain was quickly converted to discharge by rapid, shallow, subsurface runoff. Nitrate concentration remained low in channel water reflecting its low concentration in precipitation. Even by May 22, nitrate concentration in five diel samples, each from two stations, averaged only 7.5 ± 1.3

μg/L indicating both a low, uniform concentration and dominance by quick-flow components. As precipitation continued to decrease temporally, quick-flow became a decreasing proportion of discharge while water of longer residence time increased proportionally. By late summer, surface soils were very dry and discharge consisted almost entirely of groundwater.

Groundwater transport across the groundwater–stream water interface was controlled by a dynamic balance between hydrologic head on the hillslope and lateral exchange with the channel. In spring, when the water table was high, lateral mixing of oxygen-saturated water was extensive and highly aerobic habitats dominated the hyporheic zone (Table 1). Even at lowest summer flows, lateral exchange of channel water was extensive (Bencala 1984; Triska et al. 1989b; K. E. Bencala, pers. comm.) and aerobic habitats extended several metres inland (Table 1). At sites where exchange of channel and hyporheic water was minimal (i.e. WB2 and WC4) poorly-oxygenated conditions dominated even during spring. April through October, discharge decreased, the water table lowered, water temperatures rose, and the photoperiod increased then gradually decreased. The interaction of these local physical/chemical properties resulted in (1) a high diel nitrate demand for channel production, (2) an oxidation-reduction gradient laterally between the channel and the inland hyporheic zone, and (3) higher nitrate concentration at the base than at the head of the reach.

High nitrate demand occurred in late spring through autumn. May samples indicated low nitrate concentration with little diel variation. As summer approached, discharge decreased so that algal uptake dominated nitrate retention in the channel during daylight hours. Both the patterns and magnitude of midday decrease in concentration were similar to those observed at the same site in 1979 (Triska et al. 1989a). High daylight uptake of nitrate has also been reported by Gregory (1980). He found up to 80% midday decrease from midnight concentrations, depending on stream order and canopy cover. Uptake by photoautotrophs is not the only nitrate retaining process. Flume studies at Little Lost Man Creek (Triska et al. 1983) and nitrate amendments to the channel (Triska et al. 1989a) both indicate up to 20% night uptake by algae, heterotrophic components of epilithon, and possibly denitrification. Thus, at summer, base flow biotic demand is a major sink for transported nitrate.

An oxidation-reduction gradient from the channel to inland hyporheic sites occurred along all transects. At the alder transect, dissolved oxygen was detected even at our most inland well during May whereas water at WB2, WC4, and WD3 seemed almost continually depleted of oxygen. We conclude that dissolved oxygen gradients result from hydrodynamic processes in conjunction with biotic demand. Chloride data of K. E. Bencala (pers. comm.) indicate that the highly oxic habitats coincide with exchange flows between the channel and hyporheic zone. Conversely, poorly oxygenated zones such as WB2, WC4, and WD3 seem almost continually isolated from effective hydrologic exchange with the channel. The mechanism is presumably advection of oxygen-saturated water from the wetted channel driven by localized pressure distributions along the coarse bed (Bencala 1983; Bencala 1984; Savant et al. 1987; Thibodeaux and Boyle 1987) although thermal convection is also possible in sandy sediments (Whitman and Clark 1982). In either case, dissolved oxygen in the hyporheic zone was supplied by exchange with the channel rather than by input of upslope groundwater.

While the chloride data indicate exchange flow was not effective to furthermost inland wells (K. E. Bencala, pers. comm.), measurable DO was observed at all well locations. As a result, the oxidation-reduction gradient did not span the full range of redox potential indicating that obligately anaerobic processes were a minor component of hyporheic metabolism. Processes such as sulfate reduction or methanogenesis would be confined to anaerobic microsites or zones operationally defined as groundwater.

Nitrate concentration in wells was typically higher than channel water when well DO was >3.0 mg/L (except WA4 in July and August), and was lower than channel water at lower oxygen concentrations. Conversely, ammonium was typically <10 μg/L in wells when DO exceeded 3.0 mg/L and was higher in wells with less oxic conditions. Under nearly anoxic conditions ammonium concentration exceeded 1.0 mg/L at WC4. We conclude that DIN input across the groundwater–stream water interface consisted primarily of ammonium and that during transport to the channel was oxidized to nitrate.

Nitrification in the hyporheic zone explains higher nitrate output at the base of the reach than input at the head despite biotic uptake along the channel. The Rivulet constituted a minor nitrate source to the channel, since its contribution to discharge was insignificant. Increased nitrate concentration downstream is contrary to most published reports. However, many of these studies were conducted in water with high anthropogenic nutrient inputs from agriculture (Kaushik and Robinson 1976; Hill 1981, 1983, 1988) or forestry (Swank and Caskey 1982) compared to the oligotrophic waters of Little Lost Man Creek. Equivalent nitrate perturbation to Little Lost Man Creek similarly converted the reach to a net consumer of nitrate (Triska et al. 1989a).

The capacity of nitrification to impact surface water chemistry is dependant on DO and ammonium availability, and maintainance of a suitable bacterial population. At Little Lost Man Creek oxygen was available in the hyporheic zone in conjunction with exchange of flows, and ammonium was available in groundwater. Under the typical summer flow regime, oxygen concentration was sufficient to support nitrification at all locations penetrated by stream water. Keeney (1973) reports that dissolved oxygen concentrations as low as 0.3 mg/L sustain the process. In the vicinity of the oxic–anoxic interface, hydrologic transport of dissolved oxygen typically limits nitrification rates (Greenwood 1962).

Higher nitrification potential in sediments from the channel than WA2 or WA3 was unexpected since nitrifiers grow slowly (Focht and Verstraete 1977; Cooper 1983), are susceptible to scouring (Cooper 1983), and subject to invertebrate grazing. Apparently the long interval between spates during summer permitted surface populations of nitrifying bacteria to become established.

A mechanism similar to the channel presumably regulated ammonium oxidation in the tributary (Rivulet). In the tributary, surface water had high DO and low ammonium levels (<10 μg/L), whereas in the groundwater of the tributary (WB2) oxygen concentration was low and ammonium high (>30 μg/L). Atmospheric aeration of surface water in the Rivulet in conjunction with emerging groundwater contributed jointly in the biological oxidation of ammonium to nitrate in the Rivulet.

Integrating background water chemistry with the results of our process studies indicated a highly interactive channel-hyporheic system with predictable patterns of process within

the reach. The increase in channel nitrate was consistent with the annual summer decrease in precipitation and increase in nitrification rates in channel sediments between August and October. We hypothesize that nitrate production in the subsurface was controlled primarily by dissolved oxygen supplied by hydrologic exchange between channel and hyporheic waters, and secondarily by ammonium concentration. Slurries of aerobic sediments typically demonstrated nitrate production even if unamended with ammonium, although high ammonium amendment further stimulated the process, possibly by saturating both the enzymatic capacity of nitrifying bacteria and the sorbtive capacity of the sediments. The results were different than a purely Michalis–Menten kinetic response expected from culture studies, since the slurries represented both a community response and interaction with the lithology of the sediments. Nitrate production under background ammonium concentration indicated that either saturation occurred at extremely low ammonium concentration or ammonium exchange with sediments was involved. Ammonia is readily sorbed and exchanged on clay minerals and clay and humic complexes (Boatman and Murray 1982). We hypothesize that sediment sorption sequestered amended ammonium over the course of the experiment, controlling availability by exchange with interstitial water. As a result, nitrate production was an interactive result of both biotic and lithologic conditions. Under natural conditions sorption and exchange can provide an ammonium pool much larger than that assayed from interstitial water (Richey et al. 1985). We hypothesize that under summer base flow conditions the limiting step in nitrate production at oxic sites was the chemical equilibrium between bacterially colonized sediment and interstitial water. Once desorbed, interstitial ammonium was oxidized by autotrophic nitrification and then readily leached into hyporheic flows for either biological assimilation or dissimilatory reduction if transported to anoxic microsites (Duff and Triska 1990).

Slurries from nearly anoxic groundwater zones lacked nitrification potential under all conditions, and often exhibited nitrate demand even when incubated under aerobic conditions. Thus the temporally shifting oxic hyporheic zone was the site of nitrate production. The groundwater–stream water interface shifted toward the channel in summer as decreasing discharge limited lateral exchange of oxygen with the hyporheic zone. In response, nitrification generally decreased from bankside toward the groundwater–stream water interface. Nitrification potential was highest draining an area clearcut in 1965, while nitrification rates in the gravel bar wells and old-growth transect were lower but comparable. The overall pattern of the process was predictable both spatially and temporally despite the wide variety of habitats sampled.

Heterotrophic nitrification was insignificant at two locations studied, although DON was a major component of theoretically available nitrogen. At WB2, DON did decrease throughout August, and had higher concentration than the aerobic hyporheic zone, perhaps indicating some heterotrophic consumption. Most DON may have become refractory before reaching the aerobic hyporheic zone. Gjessing (1976) has reported that nitrogen content of aquatic humus is typically lower than soil humus. Finally, Keeney (1973) maintains that heterotrophic oxidation in nature is negligible compared to autotrophic. In the absence of further studies we conclude that heterotrophic nitrification is an insignificant source of nitrate.

The field study confirmed the occurrence of nitrification and provided confirmation of ammonium sorption to sediments. The increase in nitrate concentration was significant relative to background even under conditions of rapid flow through the lateral hyporheic zone and indicated high nitrification potential in natural subsurface flows. The concentration increase was especially significant since subsurface flow through WC3 was <3 h travel time. Desorption for 6 d following cutoff showed that sorbed ions were exchangeable with interstitial flows and provided evidence of control of ammonium availability under natural hydrodynamics.

Comparisons between various approaches indicate that nutrient flux in streams can be a complex local interaction of biotic processes in the channel and in hyporheic interstices, exchange reactions on sediment surfaces and channel hydrodynamics. While our assays were lateral from the channel, presumably identical processes occur vertically through the hyporheic zone (Triska 1989b). Such process interaction is probably more common than suspected, but the magnitude of nutrient flux will vary depending on the amount of anthropogenic nitrogen input, and hydrologic permeability of the bed which determines the depth and lateral extension of suitable hyporheic habitat.

Acknowledgements

We thank K. E. Bencala and G. W. Zellweger for help in the installation of wells, and in stream experiments. We also thank C. M. Pringle, C. N. Dahm, and two anonymous reviewers for reading the manuscript and providing numerous beneficial suggestions.

References

ARMSTRONG, F. A. J., AND S. TIBBITS. 1968. Photochemical combustion of organic matter in seawater, for nitrogen, phosphorus and carbon determination. J. Mar. Biol. Assoc. UK. 48: 143–152.

ARMSTRONG, F. A. J., P. M. WILLIAMS, AND J. D. M. STRICKLAND. 1966. Photo oxidation of organic matter in sea water by ultraviolet radiation, analytical and other applications. Nature (Lond.) 211: 481–488.

BENCALA, K. E. 1983. Simulation of solute transport in a mountain pool-and-riffle stream with a kinetic mass transfer model for sorption. Water Resour. Res. 19: 732–738.

1984. Interactions of solutes and streambed sediment. 2. A dynamic analysis of coupled hydrologic and chemical processes that determine solute transport. Water Resour. Res. 20: 1804–1814.

BOATMAN, C. D., AND J. W. MURRAY. 1982. Modeling exchangeable NH_4^+ adsorption in marine sediments; process and controls of adsorption. Limnol. Oceanogr. 27: 99–110.

BRETSCHKO, G. 1981. Vertical distribution of zoobenthos in an alpine brook of the Ritrodat–Luntz study area. Verh. Int. Ver. Limnol. 21: 873–876.

COLEMAN, M. J., AND H. B. N. HYNES. 1970. The vertical distribution of the invertebrate fauna in the bed of a stream. Limnol. Oceanogr. 15: 31–40.

COOPER, A. B. 1983. Effect of storm activity on benthic nitrifying activity. Appl. Environ. Microbiol. 46: 957–960.

CUMMINS, K. W. 1975. Macroinvertebrates, p. 170–198. *In* M. A. Whitton [ed.] River ecology. University of California Press, Berkeley, CA.

DUFF, J. H., AND F. J. TRISKA. 1990. Denitrification in sediments from the hyporheic zone adjacent to a small forested stream. Can. J. Fish. Aquat. Sci. 46: 1140–1147.

FOCHT, D. D., AND W. VERSTRAETE. 1977. Biochemical ecology of nitrification and denitrification, p. 135–214 *In* M. Alexander (ed.) Advances in microbial ecology I. Plenum Press, New York, NY. 268 p.

FORD, T. E., AND R. J. NAIMAN. 1989. Groundwater–surface water relationships in boreal forest watersheds: dissolved organic carbon and inorganic nutrient dynamics. Can. J. Fish. Aquat. Sci. 46: 41–49.

GJESSING, E. T. 1976. Physical and chemical characteristics of aquatic humus. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. 120 p.

GREENWOOD, D. J. 1962. Measurement of microbial metabolism in soil, p. 138–157. *In* T. R. G. Gray and D. Parkinson (eds.) The ecology of soil bacteria. University of Toronto Press, Toronto, Ont.

GREGORY, S. V. 1980. Effects of light, nutrients and grazing, on periphyton communities in streams. Ph.D. dissertation. Oregon State University, Corvallis, OR.

GRIMM, N. B., AND S. G. FISHER. 1984. Exchange between interstitial and surface water: implications for stream metabolism and nutrient cycling. Hydrobiologia 111: 219–228.

HILL, A. R. 1981. Nitrate-nitrogen flux and utilization in a stream ecosystem during summer flows. Can. Geogr. 3: 225–239.

1983. Nitrate-nitrogen mass balances for two Ontario rivers, p. 457–477. *In* T. D. Fontaine, III and S. M. Bartell [ed.] Dynamics of lotic ecosystems. Ann Arbor Sciences Publishers, Ann Arbor, MI. 494 p.

1988. Factors influencing nitrate depletion in a rural stream. Hydrobiologia 160: 111–122.

HYNES, H. B. N. 1974. Further studies on the distribution of stream animals within the substratum. Limnol. Oceanogr. 19: 92–99.

1983. Groundwater and stream ecology. Hydrobiologia 100: 93–99.

HYNES, H. B. N., D. D. WILLIAMS, AND N. E. WILLIAMS. 1976. Distribution of the benthos within the substratun of a Welsh mountain stream. Oikos 27: 307–310.

IWATSUBO, R. T., AND R. C. AVERETT. 1981. Aquatic biology of the Redwood Creek and Mill Creek drainage basins. Redwood National Park, Humboldt and Del Norte Counties, U.S. Geological Survey Open-File Report 81–143, 115 p.

IWATSUBO, R. T., K. M. NOLAN, D. R. HARDEN, G. D. GLYSSON, AND R. J. JANDA. 1975. Redwood National Park Studies, data release number 1, Redwood Creek, Humboldt County, California. September 1, 1973–April 10, 1974. U.S. Geological Survey Open-File Report. 120 p.

KAUSHIK, N. K., AND J. B. ROBINSON. 1976. Preliminary observations on nitrogen transport during summer in a small spring fed Ontario stream. Hydrobiologia 49: 59–63.

KEENEY, D. R. 1973. The nitrogen cycle in sediment-water systems. J. Environ. Qual. 2: 15–29.

LABAUGH, J. W. 1986. Wetland studies from a hydrologic perspective. Water Resour. Bull. 22: 1–10.

LIKENS, G. E. 1984. Beyond the shoreline: a watershed ecosystem approach. Verh. Int. Ver. Limnol. 22: 1–22.

LOWRANCE, R. R., R. A. LEONARD, L. E. ASMUSSEN, AND R. L. TODD. 1985. Nutrient budgets for agricultural watersheds in the southeastern coastal plain. Ecology 66: 287–296.

LOWRANCE, R. R., R. L. TODD, AND L. E. ASMUSSEN. 1984. Nutrient cycling in an agricultural watershed: I. Phreatic movement. J. Environ. Qual. 13: 22–27.

MANNY, B. A., M. C. MILLER, AND R. G. WETZEL. 1971. Ultraviolet combustion of dissolved organic nitrogen compounds in lake waters. Limnol. Oceanogr. 16: 71–85.

PETERJOHN, W. T., AND D. L. CORRELL. 1984. Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecology 65: 1466–1475.

RICHEY, J. S., W. H. MCDOWELL, AND G. E. LIKENS. 1985. Nitrogen transformations in a small mountain stream. Hydrobiologia 124: 129–139.

SAVANT, S. A., D. D. REIBLE, AND L. J. THIBODEAUX. 1987. Convective transport within stable river sediments. Water Resour. Res. 23: 1763–1768.

SCHIMEL, J. P., M. K. FIRESTONE, AND K. S. KILLHAM. 1984. Identification of heterotrophic nitrification in a Sierran forest soil. Appl. Environ. Microbiol. 48: 802–806.

STANFORD, J. A., AND J. V. WARD. 1988. The hyporheic habitat of river ecosystems. Nature (Lond.) 335: 64–66.

STROMMER, J. L., AND L. A. SMOCK. 1989. Vertical distribution and abundance of invertibrates within the sandy substrate of low-gradient headwater stream. Freshwater Biol. 22: 263–274.

SWANK, W. T., AND W. H. CASKEY. 1982. Nitrate depletion in a second-order mountain stream. J. Environ. Qual. 11: 581–584.

THIBODEAUX, L. J., AND J. O. BOYLE. 1987. Bed-form generated convective transport in bottom sediment. Nature (Lond.) 325: 341–343.

TRISKA, F. J., V. C. KENNEDY, R. J. AVANZINO, AND B. N. REILLY. 1983. Effect of simulated canopy cover on regulation of nitrate uptake and primary production by natural periphyton assemblages, p. 129–160. *In* T. D. Fontaine and S. M. Bartell [ed.] Dynamics of Lotic Ecosystems. Ann Arbor Science, Ann Arbor, MI. 494 p.

TRISKA, F. J., V. C. KENNEDY, R. J. AVANZINO, G. W. ZELLWEGER, AND K. E. BENCALA. 1989a. Retention and transport of nutrients in a third order stream: channel processes. Ecology 70: 1877–1892.

1989b. Retention and transport of nutrients in a third-order stream: hyporheic processes. Ecology 70: 1893–1905.

WHITMAN, R. L., AND W. J. CLARK. 1982. Availability of dissolved oxygen in interstitial waters of a sandy creek. Hydrobiologia 92: 651–658.

WILLIAMS, D. D. 1989. Towards a biological and chemical definition of the hyporheic zone in two Canadian rivers. Freshwater Biol. 22: 189–208.

WILLIAMS, D. D., AND H. B. N. HYNES. 1974. The occurrence of benthos deep in the substratum of a stream. Freshwater Biol. 4: 233–256.

Distributional Differences and Population Similarities for Two Deep-Sea Crabs (Family Geryonidae) in the Northeastern Gulf of Mexico[1]

F. D. Lockhart and W. J. Lindberg[2]

Department of Fisheries and Aquaculture, University of Florida, 7922 nw 71st Street, Gainesville, FL 32606 USA

N. J. Blake and R. B. Erdman

Department of Marine Science, University of South Florida

and H. M. Perry and R. S. Waller

Gulf Coast Research Laboratory

Lockhart, F. D., W. J. Lindberg, N. J. Blake, R. B. Erdman, H. M. Perry, and R. S. Waller. 1990. Distributional differences and population similarities for two deep-sea crabs (family Geryonidae) in the northeastern Gulf of Mexico. Can. J. Fish. Aquat. Sci. 47: 2112–2122.

For golden crab, *Chaceon fenneri*, and red crab, *Chaceon quinquedens*, numbers per trap, sex, and crab size were tested for broad bathymetric, geographic, and seasonal patterns on the upper continental slope, northeastern Gulf of Mexico. Red crab occupied only the deepest of three sampled depths (i.e. 677 m vs. 494 and 311 m) while golden crab predominated at the upper two. Golden crab occurred adjacent to peninsular Florida, but not along the northern Gulf slope, while red crab occurred across the geographic arc sampled. Relative abundance increased southward for golden crab and northwestward for red crab, while the proportion of females increased counter-clockwise within the sampled range of each species. Golden crab exhibited a *Chaceon*-typical bathymetric pattern of partial sex segregation and size inversely related to depth, but male and female bathymetric patterns shifted seasonally with lags between geographic areas. Interspecific competition cannot explain the species depth zonation, while geographic and seasonal patterns may relate to Loop Current–Florida Current circulation.

On a procédé pour deux crabes, *Chaceon fenneri* et *Chaceon quinquedens*, à des enquêtes sur le nombre de crabes par casier, leur sexe et leur taille afin de déterminer leur répartition bathymétrique, géographique et saisonnière sur la partie supérieure de la pente continentale, dans la partie nord-est du golfe du Mexique. On n'a trouvé des spécimens de *Chaceon quinquedens* qu'à la plus grande des profondeurs où des échantillonnages ont eu lieu (c'est-à-dire à 677 m par rapport 494 m et 311 m) alors que *Chaceon fenneri* était surtout présent aux deux profondeurs supérieures. La présence de *Chaceon fenneri* a été constatée dans la zone adjacente à la péninsule de Floride mais non pas le long de la pente nord du golfe alors que *Chaceon quinquedens* a été trouvé sur tout l'arc géographique où des sondages ont été effectués. L'abondance relative de *Chaceon fenneri* s'accroît vers le sud alors qu'elle augmente plutôt vers le nord-ouest pour *Chaceon quinquedens*, la proportion de femelles relevée augmentant dans le sens inverse des aiguilles d'une montre pour chaque espèce dans la zone échantillonnée. On a pu constater que *Chaceon fenneri* manifeste le comportement bathymétrique typique des *Chaceon* qui se traduit par une ségrégation partielle par le sexe et la taille en fonction inverse de la profondeur mais les comportements bathymétriques des mâles et des femelles fluctuent de façon saisonnière avec des décalages entre les zones géographiques. La concurrence interspécifique ne suffit pas à expliquer la zonation des espèces selon les profondeurs alors que les comportements géographiques et saisonniers sont peut-être liés à la circulation du courant Loop–courant de Floride.

Received October 5, 1989
Accepted May 24, 1990
(JA330)

Reçu le 5 octobre 1989
Acceptée le 24 mai 1990

Crabs of the family Geryonidae are common epibenthic inhabitants of the upper continental slope in the Atlantic and Indo-West Pacific (Manning and Holthius 1984; Manning 1990). Because select species are large and seemingly abundant, most research has concentrated on their fisheries potential (e.g. Haefner and Musick 1974; Wigley et al. 1975; Stone and Bailey 1980; Otwell et al. 1984; Wenner et al. 1987; Melville–Smith 1988). As major constituents of the benthos, however, these crabs might also reveal ecological and life history processes within this poorly understood deep-water community (e.g. Kelly et al. 1982; Sulkin and Van Heukelem 1980; Hines 1982, 1988). Two family members, the golden crab, *Chaceon* (= *Geryon*) *fenneri*, and the red crab, *C. quinquedens*, occur in the eastern Gulf of Mexico.

A general bathymetric pattern of population structure is apparent within Geryonidae, although depth ranges differ between species (for *Chaceon quinquedens*: Haefner and Musick 1974; Wigley et al. 1975, Haefner 1978; for *C. fenneri*: Wenner et al. 1987; Lindberg and Lockhart 1988; for *C. maritae*: Melville–Smith, 1983, 1985, 1988). In essence, females predominate in shallow zones of an overall depth range, while

[1]Journal series No. R-01011, Florida Agricultural Experiment Station.
[2]Author to whom correspondence should be addressed.

males occupy the entire depth range but predominate in the intermediate zones. Crab size also tends to be inversely related to depth for each species. In addition, previous sampling has not found overall 1:1 sex ratios, rather males were usually much more common than females.

In contrast, a bathymetric pattern for abundance in the family has not been as evident. Relative abundance of crabs diminishes at the ends of a species depth range, with greatest numbers at intermediate depths (Lindberg and Lockhart 1988). In the case of *Chaceon fenneri*, benthic habitat type significantly affects crab densities within those intermediate depths (Lindberg and Lockhart 1988; Wenner and Barans 1990).

Reproductive patterns differ for golden crabs (*Chaceon fenneri*) and red crabs (*C. quinquedens* and *C. maritae*). Female golden crabs show an annual reproductive cycle on a population level but not on an individual level (Erdman and Blake 1988; Erdman et al. 1989). Oogenesis and vitellogenesis occurs from May until October; mating occurs after this is complete, starting as early as August and stopping at an undetermined time (Erdman and Blake 1988; Erdman et al. 1989; Lindberg and Lockhart 1988). Mating is preceded by mate guarding and copulation occurs after a female molts (Elner et al. 1987; R. B. Erdman pers. obs.). Laval release starts in February and is complete by the end of March (Erdman and Blake 1988, Erdman et al. 1989).

In contrast, female red crabs do not appear to show a seasonal reproductive cycle although a peak winter spawning season is suggested (Haefner 1978; Melville–Smith 1987a). Oogenesis and vitellogenesis are essentially year round and mating is thought to be likewise (Haefner 1978). Larval release occurs over a much longer period than for golden crabs, from January through June (Haefner 1978).

At least two models have been proposed to explain the bathymetric pattern of distribution in the family Geryonidae. Wigley et al. (1975) proposed that juveniles settled deep and migrated up-slope with increasing age. Evidence inconsistent with part of their hypothesis was presented by Lindberg and Lockhart 1988). Lindberg and Lockhart (1988) proposed instead the larval accommodation and male-male competition hypothesis.

The premise of the larval accommodation and male-male competition hypothesis is that different selective pressures acted on each sex to produce behaviors underlying the observed bathymetric distribution of *Chaceon fenneri*. To accommodate the reproductive processes of oogenesis/vitellogenesis, larval development, and larval release, females might be expected to remain in warmer, shallower waters where all of these processes are favored (Kelly et al. 1982; Haefner 1978). Limitations on female reproduction and receptivity (Erdman et al. 1989; Hines 1988) are presumed to have fostered intense intrasexual competition. Behaviors associated with male-male competition could help explain the partial bathymetric segregation by size and sex.

Kelly et al. (1982) assembled the bathymetric patterns of *Chaceon* spp. along with their own work on larval behavior and derived a model of larval dispersal for *C. quinquedens*. Their model hypothesized a role for the Gulf Stream in larval dispersal. They hypothesized that one should be able to predict adult size and sex distribution based on these dispersal patterns. Their hypotheses have remained untested.

The only stable current system in the Gulf of Mexico is the Gulf Loop Current which penetrates from the Yucatan Straits northward to varying degrees annually and exits through the Straits of Florida (Maul 1977; Sturges and Evans 1983). Although highly variable, the minimum northward penetration usually occurs from November through March and does not extend much farther than the Straits of Florida. The maximum northward penetration occurs from July until September and can approach 29° N Latitude (Maul 1977; Sturges and Evans 1983). Circulation of the Loop Current is most prominent in the upper 500 m and can extend from 89° W longitude on the western edge to 84° W longitude on the eastern edge (Maul 1977; Hoffman and Worley 1986).

As a consequence of the bathymetric, geographic, and seasonal patterns reported here, we further modify the larval accommodation and male-male competition hypothesis (Lindberg and Lockhart 1988) to include geography, season, and patterns of oceanic circulation, as proposed by Kelly et al. (1982).

Methods

The overall sampling design involved replicate traps set at each of three depths (311 m, 494 m, and 677 m) at each of five locations in the northeastern Gulf of Mexico (Fig. 1) during each of four consecutive seasons. Depths were chosen within the known range of *Chaceon fenneri* and at the upper limit for *C. quinquedens* (Lindberg and Lockhart 1988). Reports of previous catches of *Chaceon* spp. were used to determine sampling locations (Otwell et al. 1984; National Marine Fisheries Service, unpubl. data; Lindberg and Lockhart 1988). Seasonal samples, to relate distributional patterns to reproductive cycles, were taken in May, August, and December of 1987, and in February of 1988.

Six Nielsen traps (Erdman and Blake 1988) baited with 10 kg of frozen mullet (*Mugil cephalus*) were placed approximately 75 m apart along a single ground-line at each of the three depths. The ground-line was anchored at each end but buoyed at only one. The prescribed sampling period for each trap set was 17–24 h; this variation in soaktime did not affect catch (Lindberg and Lockhart 1988).

Data collected at each trap-set included: date, depth, location, times set and retrieved, bottom temperatures (reversing thermometer), number of crabs of each species and sex per trap, and total weight per trap. During summer sampling at each station, sediment samples were taken with a modified Capetown dredge and returned to Gulf Coast Research Laboratories for analysis. Data collected from each crab included: species, carapace width (CW) and carapace length, sex, and if female, the presence of eggs or egg remnants.

SAS or PC-SAS software packages were used for all statistical analyses. Multi-dimensional analyses of variance (ANOVA) were used to test effects of season, area, and depth on patterns of crab abundance and size (CW) for each species and sex. Where necessary, computations were adjusted for differences in sample sizes. The count of crabs per trap was log transformed to achieve normality, and is henceforth referred to as count. In pairwise follow-up comparisons, mean differences were tested using Scheffe's mean separation procedure with a 5% level of significance in all cases.

Results

Physical Conditions

Bottom temperatures across all seasons and areas ranged from 10.4 to 13.5° C at 311 m, 7.7 to 12.2° C at 494 m, and 6.2 to

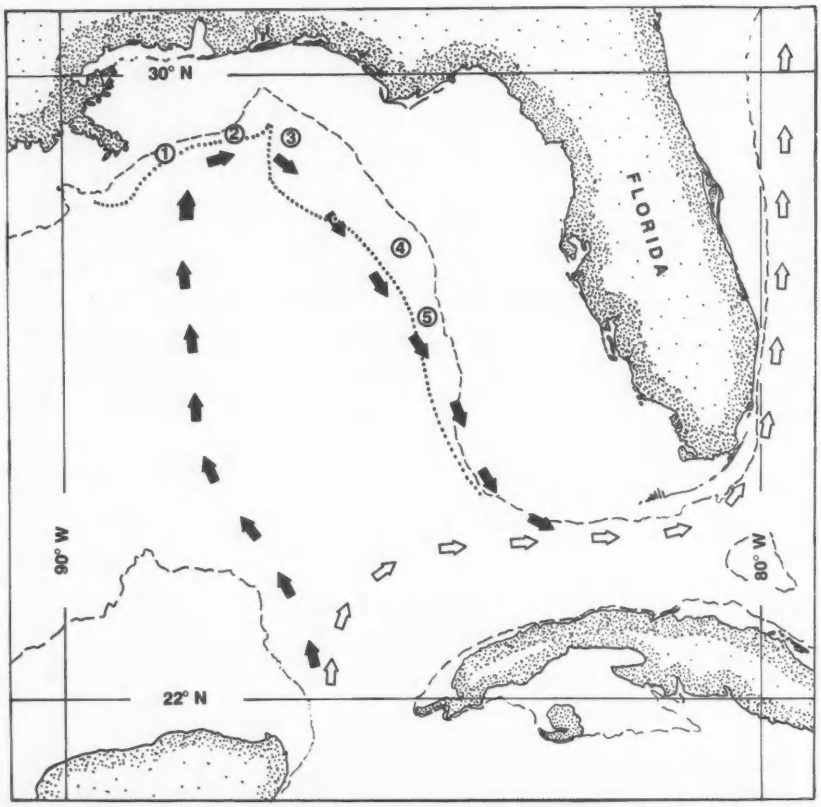

FIG. 1. Map of the eastern Gulf of Mexico with sampling areas indicated by numbered circles. Dashed line shows the 183 m isobath. Dotted line shows the 400 fathom 732 m isobath. Arrows show approximate minimum (open arrows) and maximum (closed arrows) annual penetration of the Gulf Loop Current.

TABLE 1. Composition of bottom samples with depth at each sampling area. Sediment samples are described as percent of sand/silt/clay rounded to the nearest percent.

Depth	Area 1	Area 2	Area 3	Area 4	Area 5
311 m	1/24/75	31/31/38	22/40/38	46/33/21	coral
494 m	3/32/65	23/26/51	11/89[a]	27/73[a]	76/20/4
677 m	3/32/66	3/30/67	7/93[a]	30/70[a]	64/11/25

[a]Silt and clay percentages were combined into one percentage.

9.4° C at 677 m. Season affected temperature at 311 m and 494 m, with summer and fall being warmest.

Bottom samples (Table 1) were composed for foraminiferal ooze (Doyle and Sparks 1980). Silt/clay content predominated except in area 5, where coral and sand were more prevalent. In addition, percentage of silt/clay increased with depth.

General Catch Patterns

The vast majority of *Chaceon fenneri* were caught in areas 4 and 5 (Fig. 1). Of 1919 golden crabs captured, only three were from areas 1–3, therefore only areas 4 and 5 were included in the distributional analyses for golden crabs. *Chaceon fenneri* were caught at all three depths but were most numerous at 494 m (51.2% of total). Overall, 84 and 64% of all crabs were female in areas 4 and 5, respectively. Females were trapped at all depths but most often at 311 m (45.6% of females) and 494 m (51.6% of females). Males were captured at all depths but most often at 494 m (50.3% of males). Catches varied seasonally, with greatest catches during spring (36.9% of total) and least during fall (15.7% of total).

A total of 1306 *C. quinquedens* were captured from all areas, but only from the deepest depth trapped, 677 m. Most were caught from areas 1 and 2 (75.2% of total). Female red crabs composed 79 and 66% of the catch in areas 1 and 2, respectively, as compared to 8 and 14% for areas 4 and 5, respectively. Females were caught in all areas but were most common at areas 1 and 2 (94.6% of females). Males were caught in all areas except area 3 with slightly greater numbers being captured in areas 4 and 5 (52.3% of males). Catches were greatest during winter (34.7% of total) and least during fall (5.7% of total). In addition, red crabs were completely absent from area 5 during summer and fall.

Distributional Patterns of Male Golden Crabs

Season, area, and depth affected male abundance (Table 2, Fig. 2), but because of statistical interaction, the effect of area

TABLE 2. Effects of season, depth, and geographic area on the count per trap of male *Chaceon fenneri*. Significant effects only are shown. An '×' between effects indicates interaction.

Source of variation	df	F[a] value	Results of Scheffe's comparisons
Effect of season, area, and depth (3-way ANOVA)			
Area × Depth	2	11.27***	
Season × Depth	6	4.51***	
Follow-up ANOVA: Effect of area and depth at each season (2-way ANOVA)			
Spring			
Area	1	29.95***	Area 5 > Area 4
Depth	2	20.91***	494 m > 311 m, 677 m
Summer			
Area × Depth	2	8.64**	
Follow-up ANOVA: Effect of area at each depth (1-way ANOVA)			
494 m:			
Area	1	8.8*	Area 5 > Area 4
677 m:			
Area	1	22.46***	Area 5 > Area 4
Follow-up ANOVA: Effect of depth at each area (1-way ANOVA)			
Area 5:			
Depth	2	16.08***	494 m, 677 m > 311 m
Fall			
Area	1	21.35***	Area 5 > Area 4
Winter			
Area	1	54.92***	Area 5 > Area 4
Depth	2	6.75**	494 m > 311 m

[a]Significant at 0.05 level, *; significant at 0.01 level, **; significant at 0.001 level, ***.

and depth was tested for each season. During spring, the number of males at area 5 was greater than area 4, and males were most numerous at 494 m regardless of area. During summer, area 5 had significantly more males than area 4, except at 311 m where no differences existed. In area 5, more males occurred at both 494 and 677 m than at 311 m. During fall, males were most numerous at area 5 but without depth differences in either area. During winter, males were again most numerous at area 5 but more males occurred at 494 m than at 311 m in both areas.

Depth was the only factor that had a significant effect on the size of male golden crabs ($df = 2$, $F = 3.06$, $P = 0.048$). The mean size of males at 494 m was significantly greater than at 677 m but not at 311 m (Fig. 3).

Distributional Patterns of Female Golden Crabs

Season, depth, and area affected female abundance (Table 3 and Fig. 2), but because of statistical interaction, the effect of area and depth was tested for each season. During spring, females were most abundant at 311 and 494 m rather than at 677 m, but abundance did not differ between areas. During summer, the same bathymetric pattern existed, but overall, females were more abundant at area 5. During fall, more females were at area 5 at both 494 and 677 m, but not 311 m, where no differences between areas existed. At area 4 during fall, the same depth pattern existed as during spring and summer. In contrast, females at area 5 were significantly more numerous at 494 m than at 677 m, with abundance of females at 311 m being intermediate but not significantly different. During winter, the number of females at area 5 was greater than at area 4 at both 311 and 677 m; at 494 m, no differences between areas existed. At area 4 during winter, the number at 494 m was greater than at 311 m which was greater than at 677 m; at area 5, the number at 311 and 494 m were the same and both were greater than at 677 m.

Season and depth also affected female size, but the depth effect was not consistent among seasons (Table 4, Fig. 3). During spring, summer, and fall, size was inversely related to depth. During winter, large females were located both shallow and deep (311 and 677 m) with the smallest females being intermediate in depth (494 m). The effect of season on size was significant only at 677 m where the size of females caught during winter was greater than all other seasons.

Distributional Patterns of Male Red Crabs

Red crabs were caught only at 677 m and males only in areas 1, 2, 4, and 5 so only that depth and those areas were included in the analyses. Season and area affected male red crab abundance, but with statistical interaction (Table 5, Fig. 4). At area 1, male red crabs were significantly more abundant during winter than at either spring or fall, but not summer. At area 5, red crabs were not captured during summer or fall and there were no differences in abundance between winter and spring. The effect of area on count within each season was significant only during summer when male red crabs were absent from area 5 (Table 5, Fig. 4).

Only area significantly affected male red crab size ($df = 3$, $F = 13.19$, $P = 0.0001$). Males at areas 1 and 2 were the same size and larger than in areas 4 and 5 (Fig. 5).

Distributional Patterns of Female Red Crabs

Season and area affected the count of female red crabs but with statistical interaction (Table 6, Fig. 6). The effect of season on count was significant for areas 1, 2, and 5. At areas 1 and 2, more females were caught during spring, summer, or winter than during fall. In addition at area 1, more females were caught during spring than during winter. At area 5, more females were caught during winter than during spring but females were not captured there during summer or fall.

The effect of area on count was significant for each season (Table 6, Fig. 6). During spring, the number of females caught at area 1 was greater than at area 2 which in turn was greater than all other areas. During summer, the number of females at area 1 was greater than at area 2 which in turn was greater than at area 4; females were not captured in areas 3 or 5. During fall, the number of females at area 1 was greater than at areas 2 and 4; females again were not captured in areas 3 or 5. During winter, the pattern was essentially the same as during spring with most females being found in areas 1 and 2.

Area ($df = 3$, $F = 13.19$, $P = 0.0001$) and season ($df = 3$, $F = 2.78$, $P = 0.04$) significantly affected the mean size of female red crabs. Females in area 1 were significantly larger than all other areas and those in area 2 were larger than all other areas except area 1 (Fig. 5). Females caught during fall and winter were significantly larger than those caught during spring (Fig. 5).

Discussion

Bathymetric Patterns

Our results support a *Chaceon*-typical pattern of bathymetric distribution. Females were relatively shallow with most males

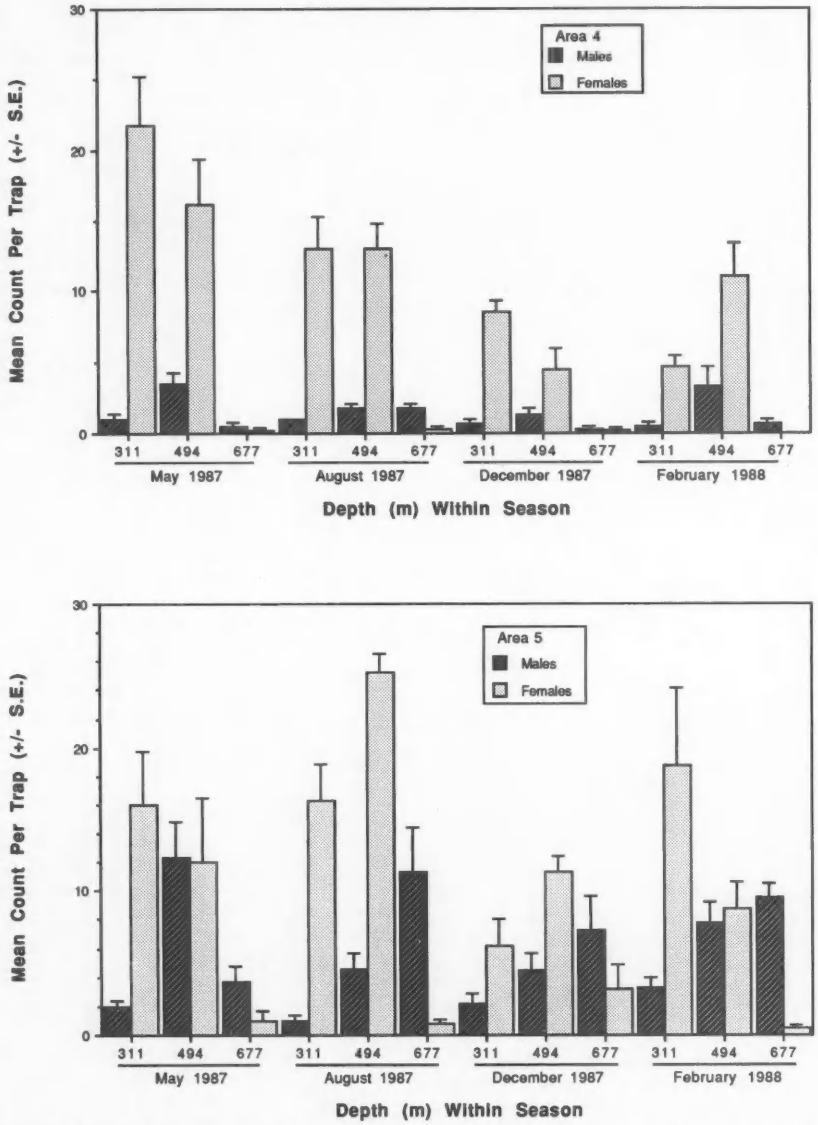

FIG. 2. Mean count per trap of *C. fenneri* at each area, depth, and season. Bars on histograms indicate standard error of the mean.

somewhat deeper. In addition, larger individuals of each sex were shallower. Important exceptions to this generalization are discussed later.

Red crabs were caught only at our deepest sampled depth which agrees with previous research (Lindberg and Lockhart 1988). This zone (ca. 677 m) appears to be an upper limit for *Chaceon quinquedens* in the eastern Gulf of Mexico. In contrast, *C. quinquedens* were caught at depths as shallow as 200 m off of New England in temperatures as high as 10–12° C (Haefner 1978). Soto (1985) attributed this shift in depth range to increased temperatures at southerly latitudes. We did not find red crabs at 494 m even though temperatures were similar to those occurring off New England (10–12° C; Haefner 1978).

Bottom type also cannot fully explain the bathymetric distribution of red crabs. Red crabs, found in situ only on soft bottom (Lindberg and Lockhart 1988), seem adapted to soft bottom habitat by their laterally flattened walking leg dactyls (Manning and Holthius 1984). In areas 4 and 5, soft bottom (silt/clay), occurred only at 677 m, coincident with red crab distribution. However, if bottom type alone determined their depth range, we should have caught red crabs shallower at areas 1–3 where soft bottom occurred at all depths. Golden crabs were not found at areas 1–3, so one cannot hypothesize that larger and presumably dominant golden crabs are excluding red crabs from more favorable, shallower depths. Neither temperature, bottom type, nor species interaction alone fully

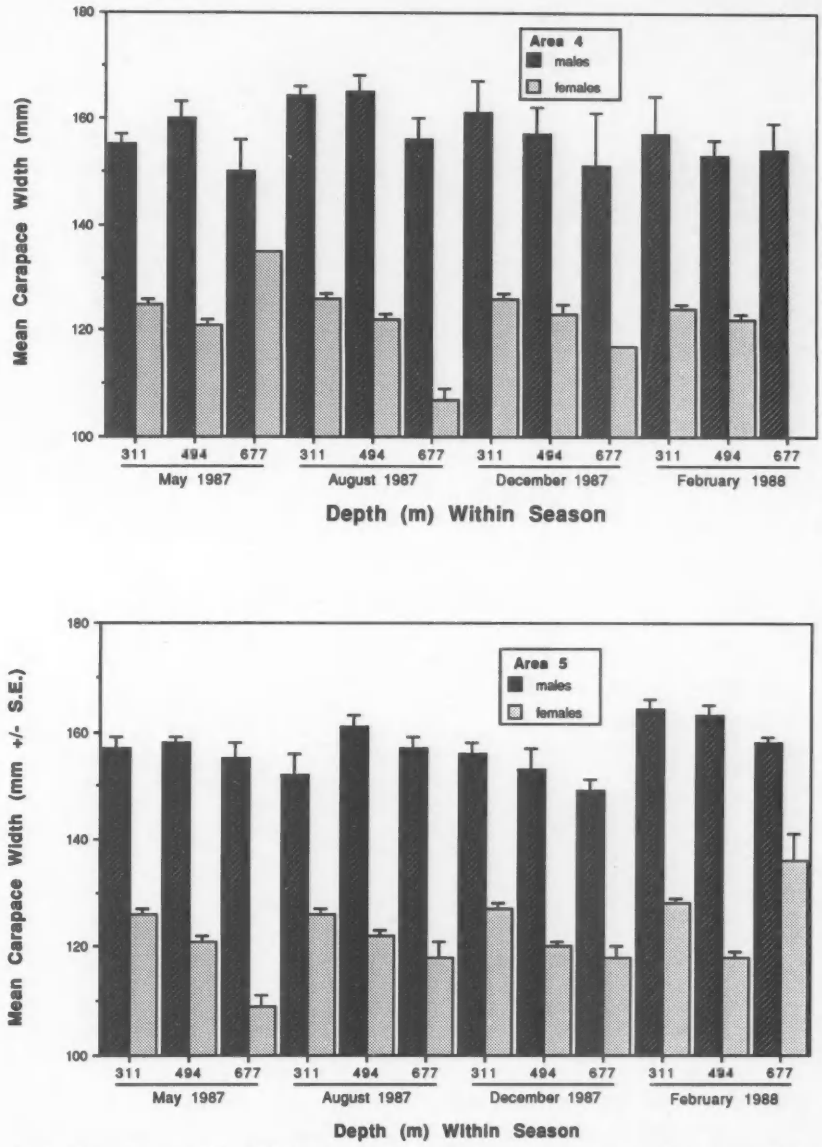

FIG. 3. Mean carapace width of *C. fenneri* at each area, depth, and season. Bars on histograms indicate standard error of the mean.

explain the differences in red crab distribution between New England and the Gulf of Mexico. Some combination of these and/or other physical factors may be responsible (Melville–Smith and Bailey 1989) or perhaps the Gulf population of red crabs is sufficiently isolated from the New England population to have different physiological tolerances.

Geographic Patterns

Golden crabs were caught predominately off peninsular Florida (i.e. areas 4 and 5), where winnowed sands, hard bottom facies, and minor submarine canyons are common at the depths occupied (Mullins et al. 1988). Golden crabs are more common on hard bottom (Lindberg and Lockhart 1988; Wenner and Barans 1990) and this habitat is lacking in areas 1–3.

Overall, golden crabs were most common at area 5, but this difference was much more pronounced for males than for females. Area 4 yielded few males whereas just less than half of the females were caught there. Consequently, the sex ratio as one moves southward, or with the prevailing surface currents, shifts toward males.

This trend in sex ratio is consistent throughout the known range of *Chaceon fenneri*. Wenner et al (1987) found less than 5% females in the South Atlantic Bight, just north of Florida.

TABLE 3. Effects of season, depth, and geographic area on the count per trap of female *Chaceon fenneri*. Significant effects only are shown. An '×' between effects indicates interaction.

Source of variation	df	F^a value	Results of Scheffe's comparisons
Effect of season, area, and depth (3-way ANOVA)			
Season × area × depth	6	4.88***	
Follow-up ANOVA: Effect of area and depth at each season (2-way ANOVA)			
Spring			
Depth	2	58.53***	311m, 494 m > 677 m
Summer			
Area	1	8.39**	Area 5 > Area 4
Depth	2	154.26***	311 m, 494 m > 677 m
Fall			
Area × Depth	2	6.38**	
Follow-up ANOVA: Effect of depth at each area (1-way ANOVA)			
Area 4			
Depth	2	29.58***	311 m, 494 m > 677 m
Area 5			
Depth	2	7.36***	494 m > 677 m
Follow-up ANOVA: Effect of area at each depth (1-way ANOVA)			
494 m			
Area	1	10.17**	Area 5 > Area 4
677 m			
Area	1	7.01*	Area 5 > Area 4
Winter			
Area × Depth	2	7.82**	
Follow-up ANOVA: Effect of depth at each area (1-way ANOVA)			
Area 4:			
Depth	2	73.02***	494 m > 311 m > 677 m
Area 5			
Depth	2	42.64***	311 m, 494 m > 677 m
Follow-up ANOVA: Effect of area at each depth (1-way ANOVA)			
311 m			
Area	1	15.57**	Area 5 > Area 4
677 m			
Area	1	5.0*	Area 5 > Area 4

[a]Significant at 0.05 level, *; significant at 0.01 level, **; significant at 0.001 level, ***.

TABLE 4. Effects of season and depth on the size of female *Chaceon fenneri*. Significant effects only are shown. An '×' between effects indicates interaction.

Source of variation	df	F^a value	Results of Scheffe's comparisons
Effect of season and depth (2-way ANOVA)			
Season × Depth	3	3.83***	
Follow-up ANOVA: Effect of depth at each season (1-way ANOVA)			
Spring			
Depth	2	24.3***	311 m > 494 m > 677 m
Summer			
Depth	2	19.5***	311 m > 494 m, 677 m
Fall			
Depth	2	15.1**	311 m > 494 m, 677 m
Winter			
Depth	2	22.6***	311 m, 677 m > 494 m
Follow-up ANOVA: Effect of season for each depth (1-way ANOVA)			
677 m			
Season	3	5.49**	Winter >Spring, Summer, Fall

[a]Significant at 0.05 level, *; significant at 0.01 level, **; significant at 0.001 level, ***.

TABLE 5. Effects of season and geographic area on the count per trap of male *Chaceon quinquedens*. Significant effects only are shown. An '×' between effects indicates interaction.

Source of variation	df	F^a value	Results of Scheffe's comparisons
Effect of season and area (2-way ANOVA)			
Season × Area	12	2.74**	
Follow-up ANOVA: Effect of season at each area (1-way ANOVA)			
Area 1			
Season	3	4.37*	Winter > Spring, Fall
Area 5			
Season	3	17.19***	Winter, Spring > Summer, Fall
Follow-up ANOVA: Effect of area at each season (1-way ANOVA)			
Summer			
Area	4	16.35***	1, 2, 4 > 5

[a]Significant at 0.05 level, *; significant at 0.01 level, **, significant at 0.001 level, ***.

The fishery off southeastern Florida reported very few females in the catch (R. B. Erdman, pers. obs.). In an area only 45 km to the south of area 5, females comprised approximately one fourth of the catch using the same gear and fishing methods as applied here (Lindberg and Lockhart 1988).

A similar geographic pattern was seen for red crabs. Males were essentially uniformly abundant among areas, with the exception that larger males were found at areas 1 and 2. Females, in contrast, were almost exclusively in areas 1 and 2. Once again, the ratio ofmales to females decreases as one moves counterclockwise among our study areas.

The almost complete absence of geryonid crabs at area 3 was unanticipated. A lack of suitable bottom type at this area probably explains the absence of golden crabs, but, the lack of red crabs is not as easily explained. Perhaps red crabs were slightly deeper than our trapping effort at area 3. Or perhaps food is limited by competition with other species. Otwell et al. (1984) noted that the bait placed in traps was quickly consumed presumably by isopods. We also observed this and, in addition, caught seemingly greater numbers of hagfish (*Myxine* spp.) at this area. Both of these are scavengers much like red crabs. Both might reach and consume food falls quicker than red crabs. Factors such as dissolved oxygen and sediment particle size might also play a role (Melville–Smith and Bailey 1989).

Seasonal Patterns

The bathymetric and geographical distribution of golden crabs varied seasonally, seemingly related to the reproductive cycle of both males and females. In spring, the sexes are segregated in the *Chaceon* typical pattern; females primarily shallow and males primarily at the intermediate depth (Haefner 1978; Wigley et al. 1975). In summer, the patterns changed for both species. Male and female depth ranges broadened such that segregation between the sexes was much reduced. Such a shift in depth distribution during the mating period is consistent

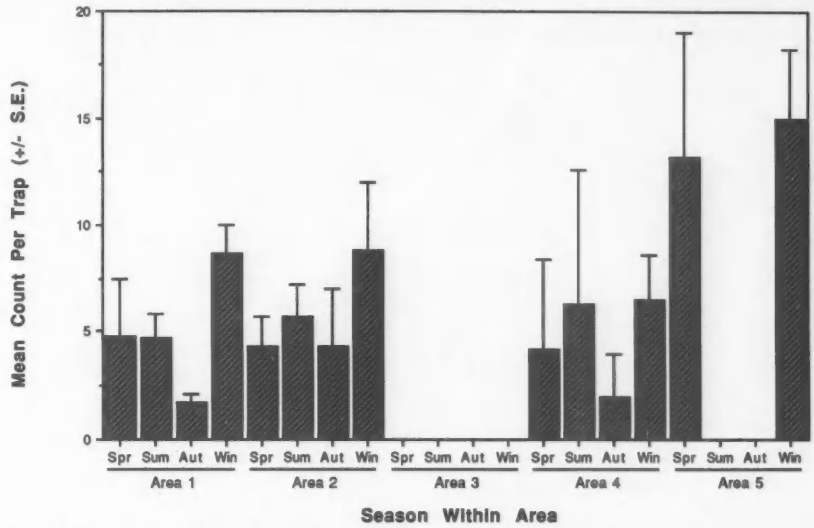

FIG. 4. Mean count per trap of male *C. quinquedens* at each season and depth. Bars on histograms indicate standard error of the mean.

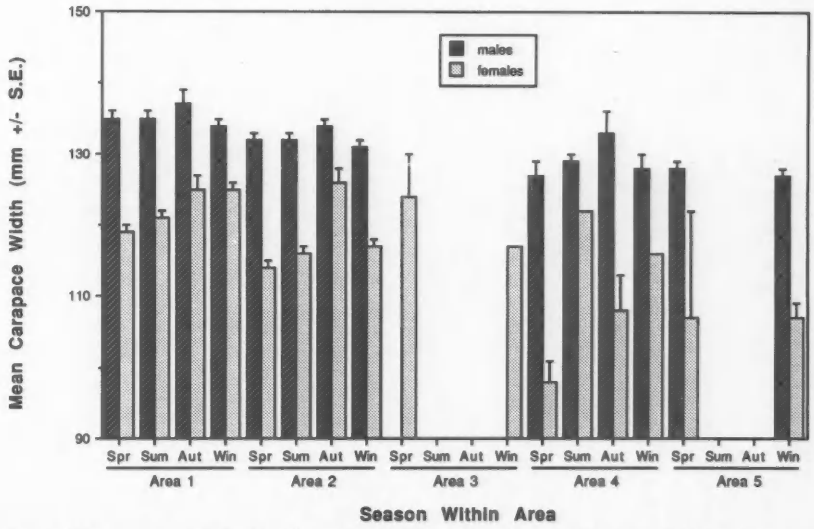

FIG. 5. Mean carapace width of male and female *C. quinquedens* at each season and area. Bars on histograms indicate standard error of the mean.

with the larval accommodation and male-male competition hypothesis (Lindberg and Lockhart 1988). Mating of *C. fenneri* has been observed in situ during late summer (Lindberg and Lockhart 1988). Our data supports Lindberg and Lockhart's (1988) hypothesis that males pair with females and carry them deeper, thus reducing competition. The overlap of sexes persisted throughout the rest of our sampling in area 5 but returned to the typical pattern in area 4 in the next season. This suggests mating might occur over a broader period in more southerly areas.

The only seasonal differences evident for red crabs were the low numbers captured during the fall and the complete lack of red crabs at area 5 during summer and fall. Only a portion of *C. quinquedens*' depth range was sampled, and therefore caution must be observed when interpreting these data. We presume red crabs were mating during this time (Haefner 1978), and undergoing a downward shift comparable to that hypothesized for golden crabs. Since we trapped at their upper depth limit, a downward movement could be reflected as low abundance in our study.

Role of the Loop Current System in Distribution and Behavior of Geryonid Crabs

The distributional patterns of *Chaceon fenneri* and possibly *C. quinquedens* in the eastern Gulf of Mexico suggest a causal

TABLE 6. Effects of season and geographic area on the count per trap of female *Chaceon quinquedens*. Significant effects only are shown. An '×' between effects indicates interaction.

Source of variation	df	F^a value	Results of Scheffe's comparisons
Effect of season and area (2-way ANOVA)			
Season × Area	12	4.74**	
Follow-up ANOVA: Effect of season at each area (1-way ANOVA)			
Area 1			
Season	3	16.75***	Spring > Winter > Fall Summer > Fall
Area 2			
Season	3	7.45**	Spring, Summer, Winter > Fall
Area 5			
Season	3	39.37***	Winter > Spring
Follow-up ANOVA: Effect of Area at each Season (1-way ANOVA)			
Spring			
Area	4	31.17***	1 > 2 > 3, 4, 5
Summer			
Area	4	82.61***	1 > 2 > 4
Fall			
Area	4	9.56***	1 > 2, 4
Winter			
Area	4	17.13***	1 > 3, 4, 5 2 > 4, 5

[a]Significant at 0.05 level, *; significant at 0.01 level, **; significant at 0.001 level, ***.

role for the Loop Current System (Maul 1977) in basic life history adaptations. Female distribution within these species' geographic ranges and the timing of larval release supports this hypothesis. Ours was the first study to discover female golden crabs in any significant numbers and was also the first to find a major population of female red crabs in the Gulf of Mexico. Both of these concentrations of females were seemingly shifted counter-current to the Loop Current circulation. We hypothesize that this counter-current shift is linked to larval release and transport, and serves to maximize recruitment into the parent population by minimizing risk of larval flushing.

Similar counter-current shifts of other female decapods have been reported or hypothesized. In the Gulf of Mexico, spawning female blue crabs (*Callinectes sapidus*) have been hypothesized to undergo a late summer spawning migration in the northeastern Gulf of Mexico that is counter to the Loop Current system (Oesterling and Adams 1979). Female western rock lobsters (*Panulirus cygnus*) are hypothesized to undergo migration to favor recruitment back into the parent population (Phillips et al. 1979). Kelly et al. (1982) proposed that only those red crab larvae (*Chaceon quinquedens*) released up-current in the species' range will recruit back into the parent population. Melville–Smith (1987a, 1987b, 1987c) in a tagging study of red crabs (*C. maritae*) off the coast of southwest Africa, showed that the only segment of the population exhibiting significant directional movement were adult females; 32% of recaptures had moved greater than 100 km and the greatest distance travelled was 380 km over 5 yr. This directional movement was later shown to be counter to the prevailing surface currents (Melville–Smith 1990). Thus, within decapods in general, and the genus in particular, adult females are capable of, and appear to undergo, long-distance directional movement in their lifetimes.

A similar migration of adult female golden crabs, counter-current to Loop Current circulation in the Gulf of Mexico (Fig. 1), would produce the geographic population structure observed off the southeastern United States. Females would be most common farthest up-current whereas males would be most common intermediate in the species geographic range. Wenner et al. (1987) reported a 15:1 (M:F) sex ratio in the South Atlantic Bight and in this study, we had an overall sex ratio of 1:4 — both consistent with hypothesized net female movements to accommodate larval retention and offset the risk of larval flushing.

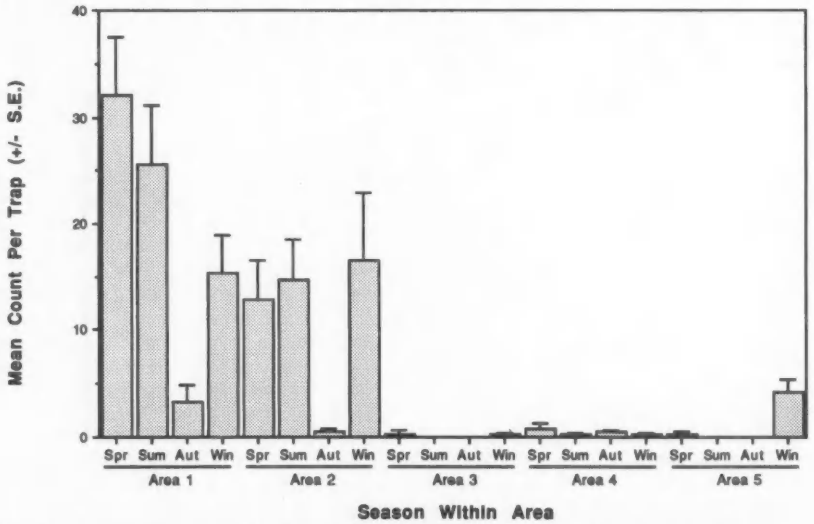

FIG. 6. Mean count per trap of female *C. quinquedens* at each season and area. Bars on histograms indicate standard error of the mean.

In fact, given this, two female strategies could maximize recruitment in a prevailing current. The first is for females to position themselves far enough up current so that entrainment would return larvae to the parent population (Sastry 1983). The second is to avoid larval entrainment altogether and thus avoid flushing of the larvae out of the system. Female *Chaceon fenneri*, and perhaps *C. quinquedens*, appear to use both strategies but rely mainly on the latter.

Female golden crabs release larvae offshore in depths usually shallower than 500 m. If larvae were released directly into the Loop Current–Gulf Stream System, they would be entrained for their entire developmental period. Given a developmental time of 33–40 d at 18°C (K. Stuck, Gulf Coast Research Laboratories, Ocean Springs, Mississippi, pers. comm.) and current speeds of 10–20 cm/sec (Sturges and Evans 1983), transport of the larvae would be 285 km to 690 km downstream. Thus, larvae released on the Atlantic side of Florida are in danger of being flushed out of the species' range before recruiting to the benthic stock. Likewise, larvae released directly into the current in the southeastern Gulf of Mexico would be flushed from the Gulf.

Female golden crabs release larvae from February to March (Erdman and Blake 1988; Erdman et al. 1989) and the greatest concentration of female golden crabs to date found in this study was in the northeastern Gulf of Mexico off central Florida. Only during this period and in this region (Maul 1977), can female golden crabs avoid complete entrainment and possible flushing of larvae out of the system. Partial entrainment of larvae might still occur, but its duration should be much reduced, and the risk of larval flushing minimal. This hypothesis predicts that most larvae should be found near the concentrations of females we found in the northeastern Gulf of Mexico with decreasing settlement further downstream. The abundance of juveniles should show a similar pattern.

One need not invoke similar counter-current movements for male geryonid crabs. In particular, males moving perpendicular to adult females (i.e. males moving up and down the continental slope) would have a greater encounter rate with females than males moving along the slope with females. Given low female reproductive frequency (Erdman et al. 1989), intense male-female competition (Lindberg and Lockhart 1988), and probability of multiple broods (Hinsch 1988) from a single protracted copulation (H. M. Perry, pers. obs.), the male strategy should be to intercept relatively rare receptive females all along the species' range, not to aggregate with presumably inseminated females. This hypothesis would predict a relatively uniform abundance of males along their geographic range. In addition, the incidence of inseminated females should be high farthest upstream with an ever decreasing percentage downstream. Our study supports the former hypothesis but we cannot address the latter.

The distributional patterns of geryonid crabs we observed are consistent with those reported from elsewhere. Furthermore, these patterns lead us to suggest that the Loop Current System has had a causal role in life history adaptations of *Chaceon fenneri* and perhaps *C. quinquedens*. In general, females are expected to release larvae during a time and in a region where risk of larval flushing is minimal (Sinclair 1988), whereas males are expected to compete intensely for rare, receptive mates.

Acknowledgements

Captain Ladnier and the crew of the R/V *Tommy Munro* were invaluable for their technical support during sampling. Thanks are extended to E. L. Wenner, and two anonymous reviewers for suggesting manuscript improvements. Sediment samples were analysed by E. G. Otvos. This work was supported by the U.S. Department of Commerce, Marine Fisheries Initiative grant numbers NA86WC-H-06135 and NA87WC-H-06142 administered through NOAA National Marine Fisheries Service and the Florida Sea Grant College Program.

References

DOYLE, L. J., AND T. N. SPARKS. 1980. Sediments of the Mississippi, Alabama, and Florida, (MAFLA) continental shelf. J. Sediment. Petrol. 50: 905–916.

ELNER, R. W., S. KOSHIO, AND G. V. HURLEY. 1987. Mating behavior of the deep-sea red crab, *Geryon quinquedens* Smith (Decapoda, Brachyura, Geryonidae). Crustaceana 52: 194–201.

ERDMAN, R. B., AND N. J. BLAKE. 1988. Reproductive ecology of female golden crabs, *Geryon fenneri* Manning and Holthius, from southeastern Florida. J. Crustacean Biol. 8(3): 392–400.

ERDMAN, R. B., N. J. BLAKE, W. J. LINDBERG, F. D. LOCKHART, H. M. PERRY, R. S. WALLER. 1989. Distributional and reproductive patterns of female deep sea golden crabs (*Geryon fenneri*) and red crabs (*G. quinquedens*) from the northeastern Gulf of Mexico, 98 p. *In* W. J. Lindberg [ed.] Fisheries development of the deep-sea golden crab, *Geryon fenneri*: geographic and seasonal production potential in the Gulf of Mexico. Final Project Report R/MARFIN-1, Florida Sea Grant College Program, University of Florida, Gainesville, FL.

HAEFNER, P. A. 1978. Seasonal aspects of the biology, distribution, and relative abundance of the deep-sea red crab *Geryon quinquedens* Smith, in the vicinity of the Norfolk canyon, western north Atlantic. Proc. Natl. Shellfish. Assoc. 68: 49–61.

HAEFNER, P. A., AND J. A. MUSICK. 1974. Observations on distribution and abundance of red crabs in Norfolk Canyon and adjacent continental slope. Mar. Fish. Rev. 36: 31–34.

HINES, A. H. 1982. Allometric constraints and variables of reproductive effort in brachyuran crabs. Mar. Biol. 69: 309–320.

1988. Fecundity and reproductive output in two species of deep-sea crabs, *Geryon fenneri* and *G. quinquedens* (Decapoda, Brachyura). J. Crustacean Biol. 8: 557–562.

HINSCH, G. W. 1988. Ultrastructure of the sperm and spermatophores of the golden crab, *Geryon fenneri*, and a closely related species, *G. quinquedens*, from the eastern Gulf of Mexico. J. Crustacean Biol. 8: 340–345.

HOFFMAN, E. E., AND S. J. WORLEY. 1986. An investigation of the circulation of the Gulf of Mexico. J. Geophys. Res. 91: 14 221–14 236.

KELLY, P., S. D. SULKIN, AND W. F. VAN HEUKELEM. 1982. A dispersal model for larvae of the deep-sea red crab *Geryon quinquedens* based upon behavioral regulation of vertical migration in the hatching stage. Mar. Biol. (Berlin) 72: 35–43.

LINDBERG, W. J., AND F. D. LOCKHART. 1988. Fisheries development of deep-sea golden crabs (*Geryon fenneri*): direct measures for stock size and population structure. Florida Sea Grant College Final Project Report R/LR-B-17, Florida Sea Grant, University of Florida, Gainesville, FL. 90 p.

MANNING, R. B. 1990. Studies on systematics of geryonid crabs, *In* W. J. Lindberg and E. L. Wenner [ed.] Geryonid crabs and associated continental slope fauna: a research workshop report. Florida Sea Grant Rep. 96.

MANNING, R. B., AND L. B. HOLTHIUS. 1984. *Geryon fenneri*, a new deep-water crab from Florida (Crustacea: Decapoda: Geryonidae) Proc. Biol. Soc. Wash. 97: 666–673.

MAUL, G. A. 1977. The annual cycle of the gulf loop current Part I: observations during a one-year time series. J. Mar. Res. 35: 29–47.

MELVILLE-SMITH, R. 1983. Abundance of deep-sea red crab *Geryon maritae* in south west African waters from photography. S. Afr. J. Mar. Sci. 1: 123–131.

1985. Density, distribution by depth of *Geryon maritae* on the northern crab grounds of south west Africa/Namibia determined by photography in 1983, with notes on the portunid crab, *Bathynectes piperatus*. S. Afr. J. Mar. Sci. 3: 55–62.

1987a. Movements of deep-sea red crab (*Geryon maritae*) off southwest Africa/Namibia. S. Afr. J. Zool. 22: 143–152.

1987b. Tagging study reveals interesting red crab (*Geryon maritae*) movements off Namibia (south west Africa). J. Cons. Int. Explor. Mer 43: 294–295.

1987c. The reproductive biology of *Geryon maritae* (Decapoda, Brachyura) off south west Africa/Namibia. Crustaceana 53: 259–275.

1988. The commercial fishery for and population dynamics of red crab *Geryon maritae* off southwest Africa, 1976–1986. S. Afr. J. Mar. Sci. 6: 79–95.

1990. *Geryon maritae* studies off South West Africa *In* W. J. Lindberg and E. L. Wenner [ed.] Geryonid Crabs and Associated Continental Slope Fauna: a research workshop report. Florida Sea Grant Rep. 96.

MELVILLE-SMITH, R., AND G. W. BAILEY. 1989. A preliminary investigation into the possible causes of depth zonation by red crab (*Geryon maritae*), off Namibia. Sel. Pap. ICSEAF int. Comm. Se. Atl. Fish. 1: 23–34.

MULLINS, H. T., A. F. GARDULSKI, E. J. HINCHEY, AND A. C. HINE. 1988. The modern carbonate ramp slope of central west Florida. J. Sediment Petrol. 58: 273–290.

OESTERLING, M. J., AND C. A. ADAMS. 1979. Migration of blue crabs along Florida's Gulf coast, *In* H. M. Perry and W. A. Van Engel [ed.] Proceedings of the blue crab colloquium. Gulf States Mar. Fish. Comm. Rep. 7.

OTWELL, W. S., J. BELLAIRS, AND D. SWEAT. 1984. Initial development of a deep-sea crab fishery in the Gulf of Mexico. Fla. Sea Grant Coll. Rep. 61, 29 p.

PHILLIPS, B. F., P. A. BROWN, D. W. RIMMER, AND D. D. REID. 1979. Distribution and dispersal of the phyllosoma larvae of the western rock lobster, *Panulirus cygnus*, in the southeastern Indian Ocean. Aust. J. Mar. Freshwater Res. 30: 773–783.

SASTRY, A. N. 1983. Pelagic larval ecology and development, p. 213–282. *In* F. J. Vernberg and W. B. Vernberg [ed.] The Biology of Crustacea, vol. 7: behavior and ecology. Academic Press, New York, NY.

SINCLAIR, M. 1988. Marine populations: an essay on population regulation and speciation. University of Washington Press, Seattle, WA. and London, UK. 252 p.

SOTO, L. A. 1985. Distribution patterns of deep-water brachyuran crabs in the straits of Florida. J. Crustacean Biol. 5: 480–499.

STONE, H., AND R. F. J. BAILEY. 1980. A survey of the red crab resource on the continental slope, N. E. Georges bank and western Scotian shelf. Can. Tech. Rep. Fish. Aquat. Sci. 977: 9 p.

STURGES, W., AND J. C. EVANS. 1983. On the variability of the loop current in the Gulf of Mexico. J. Mar. Res. 41: 639–653.

SULKIN, S. D., AND W. F. VAN HEUKELEM. 1980. Ecological and evolutionary significance of nutritional flexibility of planktotrophic larvae of the deep-sea red crab, *Geryon quinquedens*, and the stone crab, *Menippe mercenaria*. Mar. Ecol. Prog. Ser. 2: 91–95.

WENNER, E. L., AND C. A. BARANS. 1990. In situ estimates of density of golden crab, *Geryon fenneri*, from habitats on the continental slope, southeastern U.S. Bull. Mar. Sci. 46 (2).

WENNER, E. L., G. F. ULRICH, AND J. B. WISE. 1987. Exploration for golden crab, *Geryon fenneri*, in the south Atlantic bight: distribution, population structure, and gear assessment. Fish. Bull. 85: 547–560.

WIGLEY, L. G., R. B. THEROUX, AND H. E. MURRAY. 1975. Deep-sea red crab, *Geryon quinquedens*, survey off north-eastern United States. Mar. Fish. Rev. 37: 1–21.

Assessment of the Piscine Micronucleus Test as an in situ Biological Indicator of Chemical Contaminant Effects

Kenneth R. Carrasco, Karen L. Tilbury, and Mark S. Myers

Environmental Conservation Division, Northwest Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, Washington 98112 USA

Carrasco, K. R., K. L. Tilbury, and M. S. Myers. 1990. Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Can. J. Fish. Aquat. Sci. 47: 2123–2136.

The piscine micronucleus test was assessed for sensitivity and practicality as an in situ biological indicator of chemical contaminant exposure by an application to the peripheral blood of wild white croaker (*Genyonemus lineatus*) collected from polluted and reference areas along the California coast. The several types of variations from the usual erythrocyte nuclear morphology observed in the blood smears collected for this study were described and the frequencies were compared with measured levels of certain classes of contaminants in white croaker bile and liver, and in sediment collected at each site. In addition, the frequencies were also compared with the prevalence of idiopathic lesions in the livers of the croaker. However, the piscine micronucleus test appeared to lack sensitivity to the presence and effects of the measured contaminants because no association was detected between the frequencies of the erythrocyte nuclear variations and levels of chemical contamination, even though most of the observed variations resembled the published descriptions of variations presumed to be nuclear lesions in earlier investigations. Even if the test had been found to be sensitive, the low and variable frequencies of the observed nuclear lesions would present a serious difficulty to the useful application of the test.

On a évalué la sensibilité et la facilité d'utilisation comme indicateur biologique in situ de l'exposition à des contaminants chimiques de l'essai micronucléique chez les poissons en l'appliquant au sang périphérique de scènes (*Genyonemus lineatus*) capturées dans des zones polluées et des zones de références situées le long de la côte californienne. Les divers types de variations par rapport à la morphologie nucléaire habituelle des globules rouges relevées dans les frottis sanguins réunis dans le cadre de cette étude ont été décrits et l'on en a comparé les fréquences avec les niveaux mesurés de certaines catégories de contaminants dans le foie et la vésicule des scènes ainsi que dans des sédiments prélevés à chaque site. De plus, on a également comparé les fréquences avec la prévalence de lésions idiopathiques dans les foies de scènes. L'essai micronucléique chez les poissons semble insuffisamment sensible à la présence et aux effets de contaminants mesurés puisque l'on n'a décelé aucune association entre les fréquences des variations nucléaires des globules rouges et les niveaux de contamination chimique, même si la plupart des variations observées ressemblaient aux descriptions publiées des variations que l'on estimait être à l'origine de lésions nucléaires lors d'enquêtes précédentes. Même si l'on avait constaté que le test est sensible, la faiblesse et la variabilité des fréquences des lésions nucléaires observées compromettraient gravement l'utilité de son application.

Received August 18, 1989
Accepted May 15, 1990
(JA282)

Reçu le 18 août 1989
Accepté le 15 mai 1990

The mammalian micronucleus test is a well-known cytogenetic technique which assesses genotoxic damage by the visual examination of cells for chromosome fragments (Heddle et al. 1983). Although originally developed for use with the mouse (Schmid 1976), the technique was subsequently modified by Hooftman and de Raat (1982) for the application to fish in the laboratory. This modification, known as the "piscine micronucleus test," has been recently proposed as a potentially rapid and inexpensive in situ biological indicator of chemical contamination in wild fish (Hose et al. 1987) and invertebrates (Brunetti et al. 1988). In the present paper we assess the use of the piscine micronucleus test for effectiveness in determining the effects of exposure to certain classes of chemical contaminants in wild marine fish inhabiting the nearshore environment.

A micronucleus is a supernumerary nucleus visible by light microscopy in the cytoplasm of a cell (Schmid 1976). Also known as "Howell–Jolly bodies" in mammals (Schlegel et al. 1986), they are formed in any type of dividing cell when a whole or fragmented chromosome lags behind the other chromosomes during anaphase because of an aneuploidic or clastogenic event (Schmid 1975, 1976; Landolt and Kocan 1983; MacGregor et al. 1987). The enumeration of these fragments in the mammalian erythrocyte has served as an effective, relatively simple cytogenetic assay for the detection of genotoxic effects of specific chemicals in small mammals under strict, standardized laboratory conditions (Schmid 1975, 1976; Heddle et al. 1983; MacGregor et al. 1987). The technique conventionally uses the erythrocyte (Schmid 1976) because: (1) straightforward and rapid enumeration is possible since micronuclei are exceptionally conspicuous in the anucleate mammalian erythrocyte (Schmid 1976); (2) the relatively high mitotic rate of hematopoietic tissue provides a rapid response to the genotoxic effects of xenobiotics (Tice and Ivett 1985); and (3) blood smears are easily obtained and prepared. However, micronuclei in certain nucleated mammalian cells have also been utilized to quantify environmental insults in situ (e.g. Stich and Rosin 1983a).

Investigators of piscine cytogenetics became interested in the micronucleus test because it had the potential of avoiding the problems of other cytogenetic techniques such as the chromosome aberration and the sister-chromatid exchange tests. The latter procedures can effectively detect the genotoxic effects of chemical pollutants in fish (Prein et al. 1978; Hoofman 1981; Hooftman and Vink 1981), but the tests are time-consuming (Hooftman and de Raat 1982) and are not very effective with the many fish species which have relatively large numbers of small chromosomes (Hooftman and Vink 1981). Consequently, the laboratory exposure of fish to chemicals with the subsequent enumeration of micronuclei and other purportedly genotoxic lesions in the nucleated peripheral erythrocytes was undertaken by Hooftman and de Raat (1982), Manna et al. (1985), Al-Sabti (1986a, 1986b), Das and Nanda (1986), and Metcalfe (1988). The technique has also been applied to wild fish as a potential in vivo monitor of xenobiotic chemical effects by Hose et al. (1987) and to the wild mussel *Mytilus galloprovincialis* by Brunetti et al. (1988).

However, this modification of the mammalian micronucleus test for use as an in situ biological indicator in wild fish or invertebrates has not been adequately assessed for sensitivity and practicality. Interpretation of results is complicated by (1) the use of wild fish with an unknown genetic background and life history instead of laboratory-bred mammals; (2) the retention of the nucleus in the piscine erythrocyte; and (3) the different objectives and the complexities of field monitoring studies that evaluate the biological effects of a complex combination of chemical contaminants in a locality rather than the effects of a single chemical under highly controlled conditions.

Studies which have applied the micronucleus test to fish, amphibians, or invertebrates (Hooftman and de Raat 1982; Longwell et al. 1983; Manna et al. 1985; Al-Sabti 1986a, 1986b; Das and Nanda 1986; Hose et al. 1987; Spies et al. 1987; Brunetti et al. 1988; Metcalfe 1988; Long and Buchman 1989) have in common one or more of the following factors which have prohibited a rigorous appraisal of the technique as a biological indicator of chemical contamination, particularly in light of the concerns described above: (1) lack of consensus as to which irregularities of nuclear morphology may be considered genotoxic analogues of micronuclei; (2) inadequate laboratory verification that the nuclear lesions actually do result from genotoxicity or, indeed, if they result from exposure to any exogeneous agent; (3) inadequate descriptions or few photographs of the putatively genotoxic nuclear lesions that are being enumerated; (4) inconsistent methods of chemical exposure in the laboratory studies; (5) inadequate chemical analysis to confirm the presence of chemical contaminants at field sites where biological samples are collected; and (6) a paucity of samples collected from areas of intermediate pollution severity where the sensitivity of a biological indicator would be of critical importance.

We report here an empirical assessment of the piscine micronucleus test conducted to determine the usefulness of the technique as an in situ biological indicator of effects of xenobiotic exposure in fish from coastal waters. Smears of peripheral blood were collected from white croaker (*Genyonemus lineatus*) at ten localities along the California coast known to represent various degrees and types of chemical contamination. Lesions were observed which matched published descriptions of known micronuclei (Schmid 1975, 1976), in addition to other alterations of nuclear morphology which apparently have been considered by some prior experimenters to be of genotoxic origin (Hooftman and de Raat 1982; Hose et al. 1987; Metcalfe 1988). To replicate those studies, a small group of smears was scanned before actual enumeration to categorize the specific and consistent morphological variations which resembled the nuclear lesions included explicitly and implicitly in the previously published descriptions. The frequencies of the micronuclei and the other nuclear lesions were compared, singly and in several combinations, with levels of selected xenobiotic compounds in white croaker bile and liver, and in concurrently collected sediments.

The white croaker was selected as the target fish species for the present study partly because this fish is ubiquitous and is of commercial and sport fishing importance (Love et al. 1984). In addition, white croaker are believed to be sensitive to anthropogenic pollutants in the environment (Malins et al. 1987a), and the micronucleus test has already been applied to the species (Hose et al. 1987). White croaker inhabit the nearshore environment between Vancouver Island, British Columbia, Canada, and Magdalena Bay, Baja California, Mexico, in depths ranging from the surf zone to 183 m (Love et al. 1984).

Materials and Methods

Collection and Scoring of White Croaker Blood Smears

White croaker were collected along the coast of California during late June and July of 1987 with the use of launches from the NOAA Ship *McArthur*. An otter trawl of 7.5 m footrope length was deployed for hauls of 5–15 min duration.

Although white croaker were captured at many locations, ten sites were selected a posteriori as the source of specimens for this study because these sites provided an abundance of the fish while also representing a broad variety and range of severity of chemical contamination. Blood samples were taken from 13 to 15 white croaker at each of the following locations (Fig. 1): a coastal reference site at Bodega Bay; three sites inside San Francisco Bay, near Hunters Point and Redwood City, and in the Oakland Estuary; three sites in the Los Angeles Harbor complex, near Reservation Point and Long Beach, and within Cerritos Channel; a second coastal reference site at Dana Point; and two sites in San Diego Bay designated in this study as "West Harbor Island" and "North San Diego Bay." Blood was drawn from a total of 145 fish.

Upon capture, the fish were measured for total length (mm) and weight (g) before blood was drawn by caudal puncture with a heparinized 1 cc tuberculin syringe. In addition, both otoliths were extracted, gender was recorded, and a sample of liver was removed and placed in Dietrich's fixative (Gray 1954). Samples of bile from the gall bladders of a subset of captured croaker were also collected.

The drawn blood was immediately smeared on acid-washed microscope slides, allowed to air dry before fixation in absolute methanol for 5 min, and stored in dark containers until the completion of sampling. A smear from each fish was then stained with stock Giemsa solution for 45 min, cover-slipped, and randomly shuffled after coding to conceal the site of collection. Consistent varieties of anomalous nuclear morphology were categorized during a preliminary scan of 30 smears selected at random, and then the entire set of smears was scored by reading a total of 2,000 erythrocytes on each smear. This was accomplished by examining two subsets of 1,000 cells on each smear with an interim period of 1 mo and a reshuffle between examinations. The smears were examined

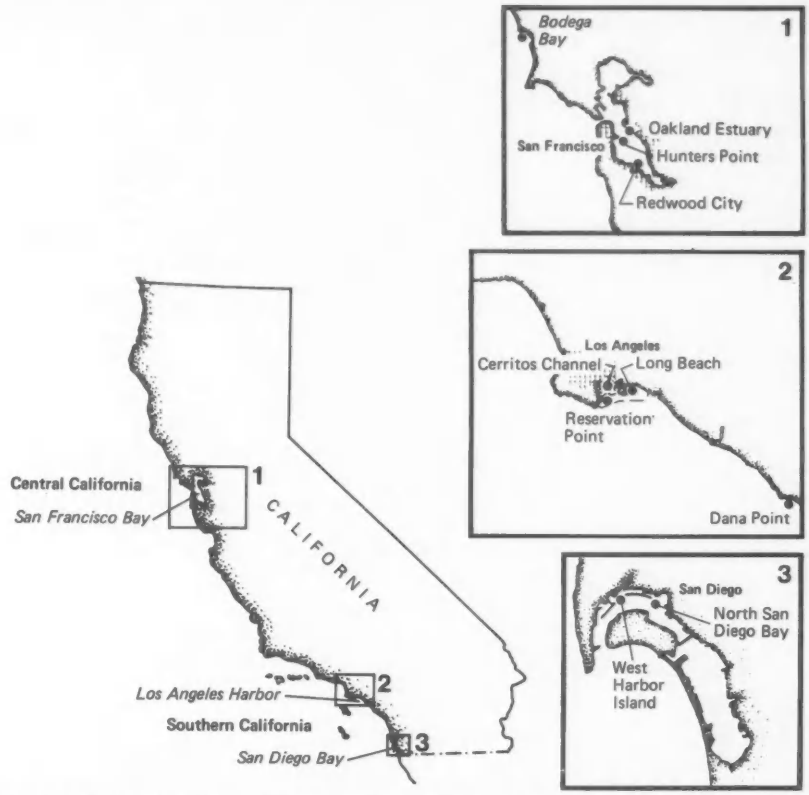

FIG. 1. Sampling site locations along the California coast. The Bodega Bay and Dana Point locations were uncontaminated reference sites.

by a single observer at high power (1000×) under oil immersion with a microscope equipped with a grid reticle.

The possibility that preparation artifacts accounted for the observed micronuclei or other nuclear lesions prompted a short ancillary study in the summer of 1988. Blood from an adult white croaker collected at Southampton Shoal in San Francisco Bay was smeared on 28 identically prepared slides which, after the application of an alteration of protocol, were subsequently stained, coded, and examined in the same manner as the original blood smears of the study. The alterations included (1) prolonged delays after smearing, up to 5 h at room temperature, before fixation; (2) a range of periods in fixative, from 5 min to 5 h; and (3) exposure to sunlight, heat, and/or marine air before fixation.

Age and Condition of Fish

Hyaline (clear) zones on both otoliths were counted under a binocular microscope as described in Kimura et al. (1979). Only the left otolith was used to assign an age to the fish in the present paper.

Fulton's condition factor was also calculated for each fish by the equation $100 \cdot w^{-1} \cdot l^{-3}$, with weight (w) in grams and length (l) in centimetres (Ricker 1975). Correction by subtraction of gonad weight (Love et al. 1984) was not considered necessary as the sampling occurred during a period of minimum gonad weight (Love et al. 1984) in the study area.

Chemical Analyses of Tissues and Sediments

Detailed descriptions of on-site collection and processing techniques of tissues and sediments are provided in Varanasi et al. (1989a).

Collection and analysis of white croaker bile

Direct tissue measurement of aromatic hydrocarbons (AHs) is not effective because these chemicals are rapidly metabolized and/or excreted after uptake (Varanasi et al. 1989b). Consequently, AH uptake by individual fish was estimated by the measurement of fluorescent metabolites in the bile using a technique devised by Krahn et al. (1984, 1986, 1987). This analysis, using HPLC/fluorescence, determined the level of fluorescent aromatic compounds (FACs) with either two or three rings (denoted in this study as FACs-L), or with four to six rings (FACs-H). One such AH is benzo[a]pyrene, a five-ring AH which can be metabolized into FACs-H that may form DNA adducts (Varanasi et al. 1986) associated with mutagenesis and clastogenesis in both mammals and fish (Hendricks et al. 1985; Thilly and Call 1986; Osborne and Crosby 1987).

All determinations of bile FACs levels from white croaker captured at a site were used to characterize that site for the degree of exposure to AHs regardless of whether blood was drawn for a smear. Medians and nonparametric statistical methods were used to describe the levels of bile FACs and to perform comparisons because (1) measures of both FACs were very het-

eroscedastic, (2) an occasional exceedingly high peak from an individual fish would bias an arithmetic mean, and (3) imprecise determinations resulted when levels of FACs were below the minimum detection level.

Collection and analysis of white croaker liver

Three composites of liver, each composite collected from ten white croaker, were analyzed from each site for summed PCBs, DDTs, and non-DDT pesticides using the techniques described in MacLeod et al. (1985). Aromatic hydrocarbons were not included because of the rapid metabolism described above. Arithmetic means of the composites were used to describe the determinations.

Collection and analysis of sediments

Sediment samples were obtained with a box corer from several stations at each collection site; three replicate grabs were performed at each station. The samples were analyzed for individual chemical contaminants using protocols cited in Krahn et al. (1988), and the concentrations of the individual chemicals were summed into an appropriate contaminant class for each station. The mean concentration and standard deviation of each class of contaminant was then calculated to characterize the sediments at that site. The classes of summed chemical contaminants included the AHs, PCBs, DDTs, and non-DDT pesticides; the specific compounds comprising each of these classes are listed in table 2 of Krahn et al. (1988), except that the dichlorobiphenyls were not included in the PCBs for the analyses presented here.

Sediment was not collected in 1987 at Cerritos Channel, but data from sediment samples collected in 1984 by Malins et al. (1987a) were used to approximate a characterization of that location. Chemical analysis of the sediments from the site had been accomplished using the techniques described in MacLeod et al. (1985) for summed PCBs, DDTs, and non-DDT pesticides. However, the AHs are not included here because the components of this contaminant class did not precisely correspond to those included in the present study.

Liver Histopathology

A histopathological examination of liver samples obtained from the same white croaker from which blood had been drawn for this study was conducted to detect idiopathic liver lesions. These lesions included (Malins et al. 1987a; Myers et al. 1987): specific degenerative/necrotic conditions (megalocytic hepatosis and hepatocellular nuclear pleomorphism), denoted in this study as "MH/NP"; severe hydropic degenerative changes in the biliary/hepatocellular epithelium; nonspecific degenerative/necrotic conditions (hepatocellular coagulative necrosis); storage disorders (fatty change and hemosiderosis); non-neoplastic proliferative conditions (hepatocellular regeneration and biliary hyperplasia); putative preneoplastic conditions (foci of cellular alteration); and neoplastic conditions (hepatocellular and/or biliary neoplasms). In general, the order in which these lesions are listed above corresponds to the hypothesized histogenetic sequence of liver neoplasm induction in fish, using wild English sole as a model (Myers et al. 1987).

The frequencies of total idiopathic liver lesions and the most common type of lesion detected were compared with the frequencies of the single and combined erythrocyte nuclear lesions.

Statistical Analyses

The frequencies of the nuclear lesions, including the micronuclei, were expressed as number per thousand (per mille). The nuclear lesions were analyzed both individually and in combinations to allow for the replication of the nuclear lesion categories utilized by prior related studies and for the analysis of groups of lesions which may have been formed by a common mechanism.

Two approaches were used for the statistical analysis of the uncombined and combined nuclear lesion data; Table 1 summarizes the statistical methodology. First, direct correlations were performed using individual fish, including comparisons between the bile metabolite data and lesion frequencies in the subset of 67 fish from which both types of data had been acquired. Second, the entire data set was separated and analyzed with respect to site by matching and correlating the nuclear lesion frequencies with the corresponding levels of environmental contaminants at the respective sampling locations.

When applicable during the statistical analyses, the critical significance level of each test was adjusted by dividing the conventional significance level ($\alpha = 0.05$) by the number of tests performed on the same data set (Miller 1981; Sokal and Rohlf 1969). This simple, conservative adjustment preserved an approximate 5% chance of committing a Type I error when multiple tests on the same data set were performed. For example, in the case of the correlations performed between the nuclear lesion data and measured parameters of environmental contamination at each site, the critical level of significance was

TABLE 1. Analyses performed on the data set. Both Tukey's and Dunn's multiple comparisons tests were modified for use with unequal sample numbers. See text for definitions of "FACs," FACs-L," and "FACs-H." Except as noted, all tests are described in Zar (1984).

Analysis of data: by individual fish
Correlations among nuclear lesions and biological parameters:
Spearman rank correlation
Correlation between bile FACs-L and FACs-H:
Spearman rank correlation
Correlations between bile FACs and nuclear lesions:
Spearman rank correlation
Analysis of data: fish grouped by site
Test for equal gender proportions:
Chi-square goodness of fit
Test for equal mean fish lengths:
Parametric ANOVA, Tukey's multiple comparison test
Test for equal mean fish ages:
Parametric ANOVA, Tukey's multiple comparison test
Relationship of length and age:
Linear regression
Test for equal condition factors:
Parametric ANOVA, Tukey's multiple comparison test
Determination of probability distribution of micronuclei and other nuclear lesions:
Dispersion test (Amphlett and Delow 1984), and variance-to-mean ratio (Elliott 1977)
Test for equal frequencies of nuclear lesions:
Kruskal-Wallis ANOVA by ranks, and Dunn's nonparametric multiple comparisons test. Also, parametric ANOVA on $\log(x+1)$ transformed data, and Tukey's multiple comparison test.
Correlations between bile FACs and nuclear lesions:
Spearman rank correlation
Test for equal liver lesion prevalence among sites:
G-test
Correlation of nuclear lesions against liver lesion prevalences:
Spearman rank correlation

TABLE 2. Sample size, mean length, mean age from otolith analysis, and mean condition factor of white croaker at each site of collection (N = 145). "Total liver lesion prevalence" is the percentage of specimens from each site with one or more of the idiopathic lesions described in Materials and Methods; MH/NP is the prevalence of megalocytic hepatosis and/or nuclear pleomorphism. Sites are listed in order of increasing mean lengths.

		Length (mm)		Mean age	Condition factor		Liver lesion prevalence (%)	
Site	n	Mean	SD	(yr)	Mean	SD	Total	MH/NP
Oakland Estuary	15	158.3	23.5	4.9	1.28	0.12	40.0	33.3
Redwood City	15	158.3	8.8	4.9	1.21	0.11	6.7	6.7
Bodega Bay	14	201.4	25.4	6.3	1.23	0.08	0.0	0.0
West Harbor Island	13	219.8	25.1	9.0	1.27	0.10	0.0	0.0
Cerritos Channel	15	222.2	23.5	7.5	1.32	0.14	53.3	53.3
Long Beach	15	231.4	15.1	7.4	1.23	0.05	13.3	0.0
Reservation Point	15	232.0	17.7	8.5	1.26	0.08	20.0	20.0
Dana Point	15	234.1	27.7	8.9	1.09	0.06	0.0	0.0
North San Diego Bay	13	236.8	31.1	10.0	1.31	0.11	0.0	0.0
Hunters Point	15	271.9	36.6	11.8	1.34	0.08	6.7	6.7

set by dividing the standard significance level (P = 0.05) by four, the number of nuclear lesion data categories tested against each individual parameter of chemical contamination. This method yielded a critical significance level (P) of 0.0125 for each correlation performed.

Results

Gender, Length, Age, and Condition of Fish

Both genders of white croaker were equally represented at each of the ten sites.

Left otoliths were analyzed from 142 of the 145 fish collected for this study; fish length (Table 2) was significantly related to age (P = 0.0001, r^2 = 0.785). The mean length of the croaker from Hunters Point was significantly larger than those from other sites and these fish were found to have a mean age of almost 12 yr, while the fish from the Oakland Estuary and Redwood City sites were significantly smaller and had a mean age estimated at about 5 yr. The mean ages of fish captured at the remaining seven sites were 6–10 yr.

The mean condition factors of the captured croaker (Table 2) were significantly different among the ten sites, but only the low mean at Dana Point was found to be distinct from the other sites. Condition factor was not related to fish length or latitude of collection site.

Morphology of Observed Erythrocyte Nuclear Lesions

Consistent variations from the normally smooth, elliptical shape of the mature erythrocyte nucleus were apparent in the white croaker blood smears collected during this study. These anomalies appeared to be similar to those described during the earlier studies which utilized the piscine micronucleus test (Hooftman and de Raat 1982; Longwell et al. 1983; Manna et al. 1985; Al-Sabti 1986a, 1986b; Das and Nanda 1986; Hose et al. 1987; Spies et al. 1987; Brunetti et al. 1988; Metcalfe 1988; and Long and Buchman 1989) and were not artifactual as they did not result from any deliberate alteration of preparation protocol during the ancillary study. Consequently, the variations were assumed to constitute lesions of the nucleus and were scored into one of the following categories during the examination of the smears. Also noted below are our suppositions as to which nuclear variations were enumerated by prior workers, except for those reported by Longwell et al. (1983) and Brunetti et al. (1988) who did not provide any description of scored lesions.

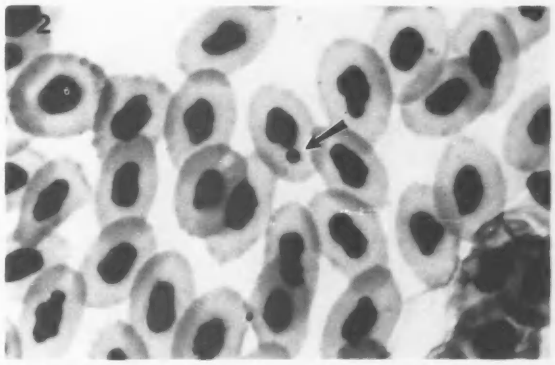

FIG. 2. Presumed micronucleus (arrow). Note connection between this structure and the main nucleus, and the variation of nuclear morphology among all red blood cells in view. Figures 2, 3, and 4 are photomicrographs of peripheral erythrocytes from a white croaker captured at Bodega Bay, an uncontaminated reference site. Giemsa-stained blood smear, 1630×.

Micronuclei (Fig. 2, 3, and 4): One or, very rarely, multiple supernumerary nuclear structures observed within the cytoplasm of an erythrocyte were scored as a single micronucleus if the following characteristics were present (Schmid 1976): a distinct outline to the structure; a round, almond, or ovoid shape; a diameter ⅕ to 1/20 of the erythrocyte; staining characteristics consistent with the chromatin of the main nucleus; and a non-refractory image. In many affected cells, the micronucleus seemed connected to the main nucleus by a very thin basophilic strand. Al-Sabti (1986a, 1986b), Das and Nanda (1986), and one of the data categories analyzed by Metcalfe (1988) apparently included only micronuclei, excluding the other nuclear variations described below.

"Blebbed" nuclei (Fig. 3): A "bleb" was a relatively small evagination of the nuclear envelope which seemed to contain euchromatin or sometimes the darker heterochromatin. Blebs

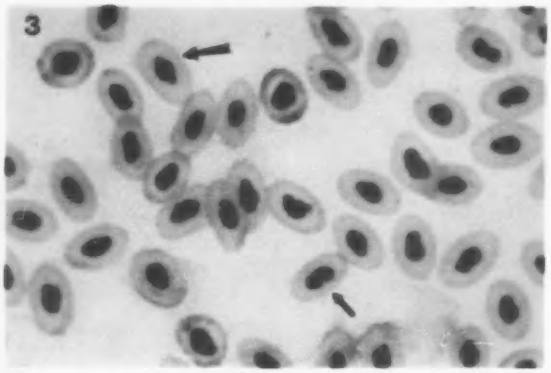

FIG. 3. "Blebbed" erythrocyte (large arrow) and a "notched" nucleus (small arrow). Note another presumed micronucleus in center of micrograph. Giemsa-stained blood smear, 1020×.

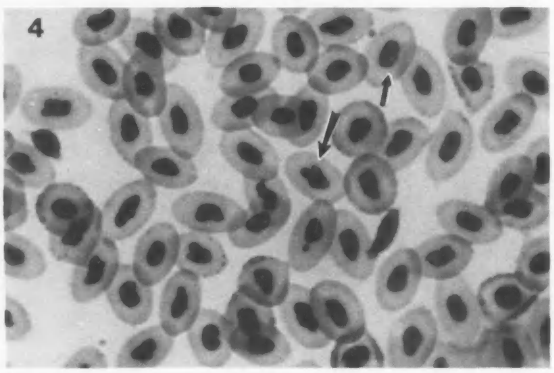

FIG. 4. "Lobed" erythrocyte nucleus (large arrow) and a "vacuole" in another nucleus (small arrow). Note a presumed micronucleus and the blebbed nucleus in nearby erythrocytes. Giemsa-stained blood smear, 1060×.

ranged in shape from a slight protrusion to a stalked structure with a completely circumscribed, round terminus closely resembling the micronuclei described just above. The size of the blebs was similar to that of the observed micronuclei.

"Lobed" nuclei (Fig. 4): Nuclei with evaginations larger than the blebs described above but without the same detailed structure were categorized as "lobed." A continuum of anomalous nuclear morphology was included in this category, ranging from simple swellings of the nuclear surface to the presence of multiple lobes forming, at times, a dumbbell shape if two lobes were present, or a clover shape if three were present. In extreme cases, the chromatin pattern seemed interrupted by a constriction at the neck of the lobe with the heterochromatin concentrated in the middle of the lobes.

"Vacuolated" nuclei (Fig. 4): A "vacuole" in a nucleus appeared as a well-defined hole devoid of any visible material. The vacuoles seemed to be demarcated by a membrane and had a relatively consistent diameter among all observations.

"Notched" nuclei (Fig. 3): A "notch" was a well-defined slit of uniform width extending to an appreciable depth into a nucleus. Notches appeared to contain no nuclear material and seemed to be demarcated by the nuclear envelope. The width of this lesion appeared similar to the diameter of vacuoles, and at times a vacuole appeared to be present at the innermost extent of a notch. The three-dimensional quality of both vacuoles and notches hindered effective photomicrography because of the thin depth-of-field at the required magnification. These lesions resembled the nuclear variations depicted in plates 55 and 58 in Ghadially (1982).

The nuclear lesions described above were grouped by summing the frequencies of the individual lesions into the following three combinations.

"M-B" lesion combination: The frequencies of the "micronuclei" and the "blebbed" nuclei were combined because a morphological continuum seemed to exist ranging from a simple bleb on the nuclear surface to a "micronucleus" attached to the main nucleus by a chromatin stalk. This combination is thought to most closely replicate the structures enumerated by Manna et al. (1985), Hose et al. (1987), and as reported by Long and Buchman (1989). The figures depicting the levels of environmental contaminants in the present paper are ordered with respect to this lesion combination because of the similarity to the lesions described in previous investigations.

"M-B-L-N" lesion combination: The "micronuclei" were combined with "blebbed," "lobed," and "notched" nuclei as all these lesions were apparently considered by Hooftman and de Raat (1982) to be of genotoxic origin, and Metcalfe (1988) analyzed all "gross irregularities of nuclear shape" as a second category in addition to micronuclei alone. In addition, this combination most closely matches the nuclear lesions reported by Long and Buchman (1989) as "nuclear pleomorphism."

"V-N" lesion combination: The "vacuolated" and "notched" nuclei were combined and analyzed separately from the other lesions as they have not been specifically mentioned by other investigators and so were possibly disregarded as artifacts. However, these lesions are suspected to be associated with aneuploidy (Ghadially 1982) as are true micronuclei (Tice and Ivett 1985).

Frequencies of Erythrocyte Nuclear Lesions and Intersite Comparisons

The micronucleus frequencies were described by arithmetic means and standard deviations as these values were exceedingly small (Table 3). The frequency distribution of this lesion type was found to assume a nonnormal distribution over the entire data set and at each of the ten sites. The other nuclear lesions and the three lesion combinations were analyzed with the use of medians and nonparametric methods as the negative binomial distribution, characterized by large variances and frequency distributions comprised of many low counts and a few extremely high counts, was fit at all ten sites.

The frequencies of several single and combined nuclear lesions were significantly correlated when considered by individual fish over the entire data set. Lesions identified as micronuclei correlated with both blebbed and lobed nuclei (both $P < 0.001$, $r_s = 0.449$ and 0.391, respectively); blebbed nuclei also significantly correlated with lobed nuclei ($P < 0.001$, $r_s = 0.555$). Vacuolated nuclei correlated with notched nuclei ($P = 0.018$, $r_s = 0.196$), lobed nuclei ($P = 0.002$, $r_s = 0.250$), and with lesion combination M-B ($P < 0.001$, $r_s = 0.337$). The V-N lesion combination correlated with lobed nuclei ($P < 0.001$, $r_s = 0.321$) and with lesion combinations M-B and M-B-L-N (both $P < 0.001$; $r_s = 0.352$ and 0.412, respectively).

TABLE 3. Observed frequencies of micronuclei and three of the nuclear lesion combinations. Data are per mille except "micronucleus prevalence" which is the percentage of smears from each site with one or more scored micronucleus. Medians of micronucleus frequency are not displayed for this cateogry as the values were exceedingly low. Sites at which white croaker were considered non-contaminated based on FACs-L and FACs-H bile metabolite chemistry are denoted with (N) and sites at which fish were found to be relatively contaminated are denoted with (C); moderately contaminated sites have no such denotation. Site sample numbers are included in Table 2.

	Micronucleus frequencies		
Site	Arithmetic mean (SD)		Prevalence (%)
Hunters Point	0.03	(0.13)	6.7
North San Diego Bay (C)	0.12	(0.22)	23.1
Dana Point (N)	0.13	(0.29)	20.0
West Harbor Island	0.15	(0.24)	30.8
Oakland Estuary (C)	0.17	(0.31)	26.7
Cerritos Channel (C)	0.27	(0.32)	46.7
Long Beach	0.39	(0.47)	53.3
Redwood City	0.45	(0.89)	46.7
Reservation Point	0.46	(0.59)	46.7
Bodega Bay (N)	0.75	(1.44)	35.7

	M-B lesion combination			
Site	Median (interquartile range)		Arithmetic mean (SD)	
Hunters Point	1.00	(0.0–2.0)	3.13	(6.93)
Dana Point (N)	1.86	(1.0–3.0)	5.65	(9.77)
Redwood City	1.98	(1.0–5.5)	3.11	(3.24)
Oakland Estuary (C)	2.00	(0.5–4.5)	3.81	(5.32)
North San Diego Bay (C)	2.50	(1.0–5.2)	3.95	(4.05)
Long Beach	2.50	(1.5–11.5)	6.20	(5.96)
Bodega Bay (N)	2.69	(0.5–6.2)	7.12	(11.80)
West Harbor Island	4.40	(2.2–7.2)	5.08	(4.17)
Reservation Point	4.98	(1.0–16.1)	10.11	(11.98)
Cerritos Channel (C)	6.50	(3.4–16.0)	10.67	(10.35)

	M-B-L-N lesion combination			
Site	Median (interquartile range)		Arithmetic mean (SD)	
Hunters Point	3.50	(2.0–5.4)	6.78	(9.84)
Dana Point (N)	4.95	(1.5–12.5)	10.29	(12.91)
North San Diego Bay (C)	5.00	(2.0–7.2)	5.87	(4.78)
Redwood City	5.40	(2.5–10.4)	8.17	(7.95)
West Harbor Island	6.50	(4.2–14.6)	8.56	(5.79)
Bodega Bay (N)	6.58	(1.8–13.4)	13.52	(20.87)
Long Beach	7.87	(6.0–16.5)	11.63	(8.88)
Oakland Estuary (C)	8.50	(4.0–14.0)	9.15	(7.12)
Cerritos Channel (C)	10.88	(6.0–27.5)	16.38	(13.32)
Reservation Point	10.96	(3.5–28.9)	15.56	(16.71)

	V-N lesion combination			
Site	Median (interquartile range)		Arithmetic mean (SD)	
Bodega Bay (N)	1.92	(0.0–2.8)	2.34	(3.69)
Oakland Estuary (C)	2.00	(1.0–3.0)	2.31	(2.00)
Dana Point (N)	2.00	(1.5–7.5)	4.38	(4.11)
Hunters Point	2.83	(0.5–4.0)	2.54	(1.86)
Redwood City	3.00	(1.0–6.0)	4.35	(4.31)
North San Diego Bay (C)	4.00	(2.0–6.0)	4.12	(2.79)
Long Beach	4.00	(1.5–7.0)	4.48	(2.76)
West Harbor Island	4.00	(3.2–6.8)	5.27	(2.58)
Cerritos Channel (C)	4.50	(2.5–7.7)	4.99	(3.64)
Reservation Point	6.00	(2.8–8.0)	6.77	(6.94)

Data analysis of the frequency of the nuclear lesions with respect to site (Table 3) yielded no significant differences among the micronucleus frequencies, the lobed nuclear lesions (both $0.10<P<0.25$), nor among the frequencies of the M-B-L-N lesion combination ($0.05<P<0.10$). The blebbed nucleus category and the lesion combination M-B were found to differ significantly among the sites (both $0.01<P<0.025$), but subsequent multiple comparison procedures were not sufficiently powerful to unequivocally discriminate which of the sites were different. The frequencies of the vacuolated nuclei and the V-N lesion combination were also significantly different among the sites (both $0.001<P<0.005$); the only significant difference found in the latter combination was between the medians of these nuclear lesions in croaker from Reservation Point and Bodega Bay.

Supplementary parametric statistical analysis of the three lesion combinations using log-transformed data, performed to more closely repilcate previous work (e.g. Hose et al. 1987), did not detect any significant differences among the sites.

Neither gender, length, nor condition factor significantly correlated with the frequency of any nuclear lesion or combination of lesions, or with the chemical measures of environmental contamination reported below. However, a significant correlation was detected between age and the frequency of vacuolated nuclei ($P = 0.005$, $r_s = 0.235$; data not shown).

Chemical Contamination in White Croaker Bile and Liver

Fluorescent aromatic compounds in bile

Bile was collected for FACs determinations from up to 15 of the white croaker captured at each site. The fish did not always have sufficient amount of bile to allow collection, but both blood smears and bile were collected from 67 (46%) of the total number of specimens.

The highest FACs-H medians (Fig. 5a) were detected in the bile of white croaker captured at the North San Diego Bay, West Harbor Island, Cerritos Channel, and Oakland Estuary sites. The bile of fish from Bodega Bay and Dana Point had the lowest FACs-H levels.

Differences among the bile FACs-L medians (Fig. 5b) were not as distinct as were the bile FACs-H values. The highest FACs-L medians were found in the bile of croaker collected at the Cerritos Channel and Oakland Estuary sites. Once again, the bile of fish captured at the Bodega Bay and Dana Point sites contained the lowest values of FACs-L.

An incidental finding was a strong correlation ($P < 0.001$; $r_s = 0.798$) between the bile FACs-H and FACs-L levels of individual fish.

Contaminants in liver

Liver composites were collected from eight of the ten sites comprising this study; liver from Hunters Point and Cerritos Channel was not analyzed. White croaker captured at Long Beach had notably high levels of all three measured contaminants (Fig. 6) in their livers, and DDT levels were also appreciable at Reservation Point. The liver composites from fish captured at Bodega Bay had the lowest levels of all three contaminants, but fish captured at Dana Point had the third highest level of DDTs among the eight sites.

Chemical Contamination of Sediments

The mean concentration of each class of sediment-associated chemical was lowest in the sediments collected at the Dana

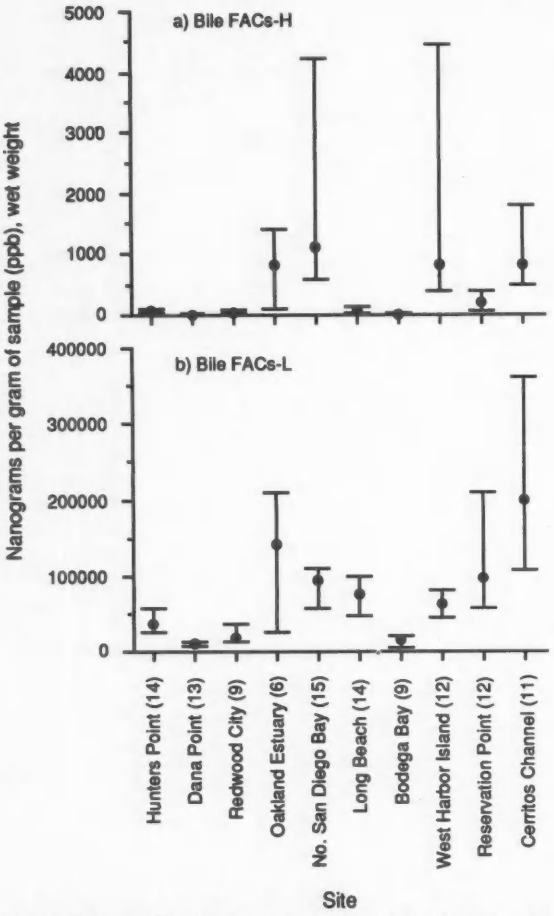

FIG. 5. Concentrations (ng/g) of FACs-H(a) and FACs-L(b) in white croaker bile collected concurrently with the blood smears used for this study. Medians are indicated by dots; bars indicate confidence limits, with confidence of 95% or greater, calculated using the binomial distribution (Zar 1984). The number of bile samples analyzed from each site is indicated in parentheses. Sites are shown in order of the medians of the M-B lesion combination (Table 3).

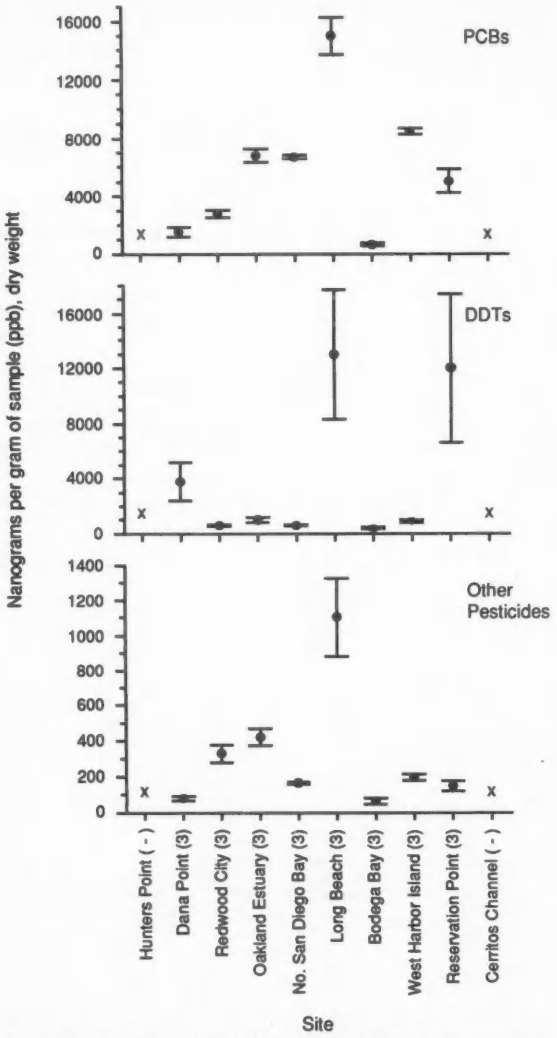

FIG. 6. Concentrations (ng/g) of chemical contaminants in composites of white croaker liver collected concurrently with the blood smears and sediments. Dots indicate means and bars indicate standard errors. Missing data are indicated in the figure by an "x."

Point and Bodega Bay sites (Fig. 7). However, a general assessment of the relative severity of sediment contamination among the other eight sites was not possible since the contribution of each chemical type to the overall contamination varied greatly among the sites.

The sediment collected at Hunters Point was the most contaminated with AHs, but appreciable levels were also found in sediment from the Oakland Estuary, North San Diego Bay, and Redwood City sites. The highest mean level of summed PCBs was found at the North San Diego Bay site. Among the pesticides, the levels of summed DDTs were highest in sediments from Reservation Point, Long Beach, and West Harbor Island. The highest level of summed non-DDT pesticides was found in sediments from the Oakland Estuary, with appreciable amounts also found at Long Beach and Redwood City.

Because the bile metabolite and sediment chemistry values were lowest at Dana Point and Bodega Bay, the white croaker collected at those sites were considered minimally exposed to chemical contaminants and the use of those locations as reference sites was supported.

Relationships Between Nuclear Lesion Frequencies and Measures of Environmental Contamination

No suggestion of any correlation was found between the FACs-H or FACs-L levels in the bile of the individual white croaker and any single nuclear lesion or combination of nuclear lesions observed in the blood smears of those fish (Table 4).

Only one correlation of significance was found with respect to site (Table 5) among the respective means or medians of any class of measured chemical contaminant and the corresponding mean or median frequency of the individual nuclear lesions or lesion combinations: between the V-N lesion combination and the summed DDTs in the sediments ($r_s = 0.80$). Correlations

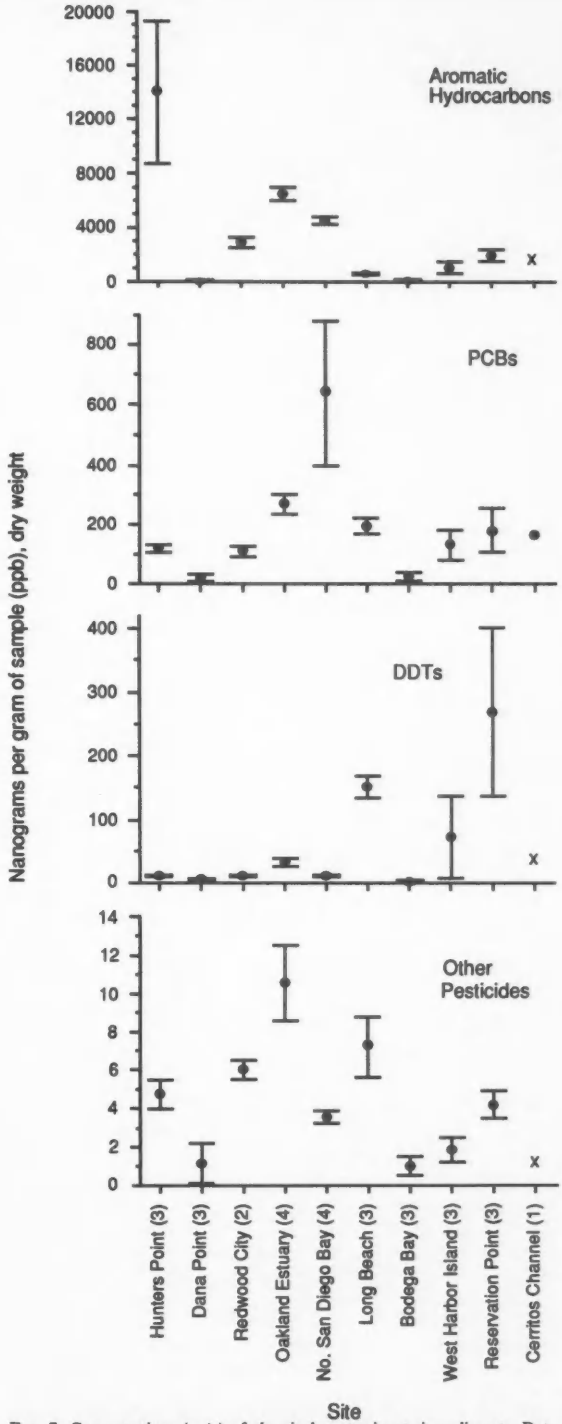

FIG. 7. Concentrations (ng/g) of chemical contaminants in sediments. Dots indicate means and bars indicate standard errors. The number of sediment sampling stations at each site is indicated in parentheses; each sample was comprised of 3 replicate grabs. Missing data are indicated by an "x."

TABLE 4. Significance levels and correlation coefficients (r_s) from Spearman rank correlations conducted between the concentrations of FACs-L and FACs-H in the bile of individual fish and the frequencies of nuclear lesions ($N = 67$).

Comparison	r_s	Significance level (P)
Bile FACs-H vs.:		
Micronuclei	−0.026	0.84
M-B lesion combination	−0.005	0.97
M-B-L-N lesion combination	−0.067	0.59
V-N lesion combination	0.012	0.92
Bile FACs-L vs.:		
Micronuclei	0.141	0.26
M-B lesion combination	0.068	0.59
M-B-L-N lesion combination	0.059	0.64
V-N lesion combination	0.086	0.49

between the medians of the M-B-L-N lesion combination and both the white croaker bile FACs-L and the total idiopathic liver lesions approached the adjusted critical significance level of 0.0125 (both $r_s = 0.70$).

Frequencies of Nuclear Lesions vs. Idiopathic Liver Lesions

Prevalences of total idiopathic liver lesions and MH/NP were significantly higher than the mean prevalence level over all of the sites only at Cerritos Channel (Table 2); the latter liver lesion was the most common among the fish captured during this study. Prevalences of both categories did not significantly correlate with the frequencies of micronuclei, the individual nuclear lesions, or with two of the lesion combinations using the adjusted P value. However, a near correlation ($r_s = 0.703$) did occur with the M-B-L-N lesion combination.

Discussion

Few statistical associations were found in this study between the frequencies of previously described nuclear lesions within white croaker erythrocytes and the levels of measured chemical pollutants in the bile or liver of the fish or in the concurrently collected sediments. This was true despite the variety of contaminants and the wide range of contaminant levels to which these fish had demonstrably been exposed.

For example, the highest mean frequency of micronuclei among the ten collection sites was found at Bodega Bay despite the low levels of measured contaminants in the bile and liver of the fish and in the sediments. Conversely, the lowest mean frequency of micronuclei was found among the fish collected at Hunters Point, despite the highest levels of sediment AHs at that site of the ten sites considered during this study. However, it should also be noted that levels of both FACs and the prevalence of liver lesions were also low at this site for this species.

The significant difference found in the M-B lesion combination between the extreme high and low sites (Cerritos Channel and Hunters Point, respectively) is of unknown importance because both the bile and the sediments were found to be relatively contaminated at both locations. Although the environmental contaminants depicted in Fig. 5, 6, and 7 are ordered with respect to this lesion combination, visual inspection does not suggest any dose-response relationship. The significant intersite difference found between the extreme high and low frequencies of the V-N lesion combination is also of unknown

TABLE 5. Significance levels (P) of Spearman correlations conducted between the mean level of contaminants measured at each site and the respective frequency of observed nuclear lesions. N indicates number of sites from which appropriate samples were collected. MH/NP denotes "megalocytic hepatosis/nuclear pleomorphism." Critical value of significance = 0.0125, as described in text. – indicates a negative correlation.

		Lesion combinations		
Comparison	Micronuclei	M-B	M-B-L-N	V-N
Sediments ($N=9$)				
Summed AHs	0.265 –	0.431 –	0.765 –	0.710
Summed PCBs	0.637 –	0.431	0.286	0.137
Summed DDTs	0.728	0.177	0.071	0.010
Summed non-DDT Pesticides	0.932 –	0.486 –	0.406	0.582
White croaker bile ($N=10$)				
FACs-H	0.200 –	0.243	0.603	0.065
FACs-L	0.960	0.093	0.025	0.049
White croaker liver ($N=8$)				
Summed PCBs	0.420 –	0.588	0.420	0.145
Summed DDTs	0.736 –	0.888	0.352	0.204
Summed non-DDT Pesticides	0.736 –	0.649 –	0.493	0.498
White croaker liver lesions ($N=10$)				
All lesions	0.473	0.433	0.023	0.238
MH/NP	0.694	0.576	0.113	0.417

importance since no trend was discernible among the other eight sites.

The near correlation between the medians of the M-B-L-N lesion combination and the respective bile FACs-L medians also does not appear to be important in light of the lack of correlation betwen these two variables on an individual fish basis, independent of site. Also, only a near correlation was observed between the nuclear lesion combination M-B-L-N and the total idiopathic liver lesions which have been shown to be reasonably reliable indicators of contaminant exposure in white croaker (Malins et al. 1987a) and in English sole (Malins et al. 1985, 1987b; Myers et al. 1987). The importance of the correlation between the V-N lesion combination medians and the summed DDTs in the sediments is unknown because this lesion category has not been previously described and the etiology is unknown. All other correlations attempted between the median frequencies of the three combinations of nuclear lesions and the levels of environmental contaminants that were measured were insignificant. Many such correlations were, in fact, negative.

Justification for the practice of combining individual nuclear lesions for statistical analysis was provided by the significant positive correlations found, in individual fish, between certain types of single and combined erythrocyte nuclear lesions. However, no inference regarding the causative mechanism may be drawn from these data.

Two possibilities may explain the lack of significant correlations between the nuclear lesions and the levels of measured contaminants: (1) the frequencies of erythrocyte nuclear lesions identified as "micronuclei" or as genotoxic analogues are not valid indicators of exposure to the particular chemical contaminants measured, or (2) the white croaker were not actually exposed to the chemical contaminants. The latter possibility must be considered during in situ sampling, but this study used various measures of contamination which reflected a variety of time scales of exposure in an effort to reduce the effects of migration by the fish; i.e. the idiopathic liver lesion prevalences and the liver tissue contaminant levels would be expected to reflect long-term exposure to contaminants, while the bile contaminant levels would indicate more short-term to intermediate time periods. As this empirical assessment of the piscine micronucleus test did not detect a relationship with any of the levels of measured contaminants, we suggest the technique has little utility as a biological indicator of toxicologically important effects of these contaminant classes. However, the possibility of a significant relationship between nuclear lesion frequencies and contaminants that were not measured in this study cannot be excluded. If the piscine micronucleus test is again considered for such a role, we feel that the agents responsible for the genesis of the erythrocyte nuclear anomalies should first be determined in the laboratory with protocols duplicating the mammalian technique and with unequivocal cytological confirmation of the actual mechanism.

Our conclusion regarding the utility of the piscine micronucleus test conflicts with the conclusions of Longwell et al. (1983), Das and Nanda (1986), Al-Sabti (1986a), Hose et al. (1987), and as reported by Long and Buchman (1989) as these workers indicated that their data supported the use of the piscine micronucleus test as a biological indicator of the exposure to chemical contamination. More guarded, but still positive, conclusions were also expressed by Hooftman and de Raat (1982), Manna et al. (1985), Brunetti et al. (1988), and Metcalfe (1988). Four factors may explain much of this inconsistency. First, very few geographic areas were compared in the field studies listed above; for example, Hose et al. (1987) compared a single highly contaminated area against a single clean reference area for each of two species. Without data from multiple sampling sites including areas of intermediate contamination, however, any relationship between the frequency of erythrocyte nuclear lesions in fish and indices of chemical contamination cannot be adequately assessed. Second, arithmetic means have often been used in other studies to quantify nuclear lesion frequencies, but occasional extremely high lesion values may severely distort the sample mean as a reflection of the true population mean. Third, results of some prior studies were difficult

to interpret because of an apparent dose-related cytotoxicity which killed the erythrocytes instead of forming a non-lethal nuclear lesion. For example, the micronucleus frequencies in the peripheral erythrocytes of *Heteropneustes fossilis* were observed by Das and Nanda (table 2, 1986) to peak at intermediate and not the highest concentrations after a 1-mo laboratory exposure to paper mill effluent. Similarly, Brunetti et al. (1988) found that two of the four mussel sampling sites in polluted areas of the Venetian Lagoon exhibited micronucleus frequencies that were lower than the two sites located at purportedly clean reference areas. Although generally optimistic about the use of the micronucleus test to monitor the marine environment for genotoxic pollutants, both papers expressed the caveat that such use would be limited to situations of sublethal contamination. Fourth, as discussed directly below, genotoxicity may have been wrongly assumed to be the causative mechanism of the lesions observed in the prior studies since other etiologies may have accounted for some proportion of the observed nuclear alterations.

It was because of the possibility of mistaking irrelevant or even normal variations of morphology for manifestations of genotoxicity that Schmid (1975, 1976) cautioned against the use of nucleated cells in the mammalian micronucleus test. Indeed, qualitative observations of the morphology of the erythrocyte nuclear lesions during the present study aroused suspicion that at least some of them were formed after, and not during, the immediately preceding mitotic division when actual micronuclei are known to be created. Although these lesions would otherwise meet all criteria for acceptance of the lesion as a micronucleus (Schmid 1975, 1976), they were visibly connected to the main nucleus by a basophilic strand or stalk of appreciable diameter. However, as a consequence of the mechanism of cytokinesis, a true micronucleus could be expected to either remain physically separated from the main nucleus or, if in close proximity, to become incorporated with the nucleus. This seems likely because the nuclear envelope forms by the fusion of free cytoplasmic vesicles against the chromatin surface of individual, free chromosomes (Lohka and Masui 1984); these enveloped chromosomes themselves then fuse to form the enveloped nucleus (Forbes et al. 1983).

Few photographs of confirmed micronuclei in poikilotherms exist. However, micronuclei photographed in the nucleated peripheral erythrocytes of amphibia by Siboulet et al. (1984), Jaylet et al. (1986a, 1986b), and Grinfeld et al. (1986) are not connected to the main nucleus. Likewise, the vast majority of micronuclei in human erythrocytes photographed before nuclear extrusion are not connected to the main nucleus. Examples of micronuclei in human blood cells which were photographed from a perspective such that the micronucleus did not overlap the nucleus and where the nucleus was not karyorrhexic include figures 130, 131, 137, and 210 in Bessis (1973); figures 1 and 2 in Schmid (1976); figure 8 of plate 14 in Platt (1979); figures 17, 26, 28, 31, 33, 54, and 55 in Castoldi (1981); plates 27, 28, 32, and 49 in Hayhoe and Flemans (1982); pages 143, 145, and 151 in Hyun et al. (1986); and figure 5-2 in Jandl (1987). In addition, nuclear anomalies described as micronuclei which were experimentally induced in exfoliated, nucleated human buccal cells have been noted to be "without any structural connection to the main nucleus" (Stich and Rosin 1984) and, in fact, photographs of such micronuclei by Stich and Rosin (1983b) and Stich et al. (1983) do not illustrate any such connection.

It is also of note that although Stich and Rosin (1983b) recommended the inclusion of all observed "nuclear aberrations" with the micronucleus frequencies, these aberrations were actually so rare during their studies that they were apparently ignored (Stich and Rosin 1983a). In contrast, in the present study the nuclear lesions which could be considered to be such "aberrations" were much more common than were the putative micronuclei.

The presence of a low-level enzootic of viral erythrocytic necrosis (VEN) within the sampled fish population is one possible explanation for the presence of nuclear lesions which resemble micronuclei and the nuclear blebs. Viral erythrocytic necrosis results in Feulgen-positive cytoplasmic inclusions which are typically basophilic (Amos 1985) and which are visually similar to the lesions observed in the present study. In fact, the presumptive diagnosis of VEN is currently based on the detection of these structures by light microscope (Amos 1985). Viral erythrocytic necrosis is widely distributed (Smail 1982) and has been confirmed or suspected in at least 20 fish species, but the disease probably occurs in many more that have not yet been examined (Post 1987). The nuclear lesions which result from VEN are often strikingly similar in appearance to the description and photographs of mammalian micronuclei. Of the numerous photomicrographs of VEN-infected erythrocytes in the literature, figure 5 of Sherburne (1973), depicting Atlantic herring (*Clupea harengus harengus*) erythrocytes infected with a disease later identified as VEN (Reno et al. 1978), is particularly similar to the lesions observed during the present study. Other relevant photomicrographs of VEN lesions are included in Appy et al. (1976), Evelyn and Taxler (1978), Smail and Egglestone (1980), Rohovec and Amandi (1981), Chen et al. (1985), Newman (1985), Leek (1987), and Post (1987). Currently, the only method for the definitive diagnosis of VEN utilizes transmission electron microscopy (Amos 1985). However, our own efforts to demonstrate the presence of these viral particles in the putative micronuclei of the white croaker erythrocytes have been unsuccessful because of the very low frequency of cells with such nuclear alterations. Clearly, some means for the quick and economical identification of VEN as the possible source of observed nuclear lesions is advisable before further development and reliable application of the piscine micronucleus test as an in situ biological indicator.

Still other mechanisms may account for some proportion of the nuclear lesions observed during this study. For example, the "lobed" nuclei observed in the erythrocytes during our study closely resemble lesions shown in photomicrographs of peripheral blood from fish with anemias induced by dietary vitamin B_{12} or folic acid deficiencies (Smith 1968; Smith and Halver 1969). Also, arsenic (Jandl 1987) and a number of drugs (Beutler 1985) are known to produce nuclear lesions in the human by interfering with DNA synthesis or with the utilization of folic acid.

Two serious problems with the piscine micronucleus test would remain if methods are ultimately developed to competently distinguish nuclear lesions resulting from genotoxic mechanisms from lesions resulting from other mechanisms. First, even if a relation had been observed between the nuclear lesions and the measured levels of contaminants during the present study, the low frequency of micronuclei and other nuclear lesions noted by ourselves and by previous studies would require the use of impractically large sample sizes to assess marginally polluted areas. For example, Long and Buchman (1989) report that among samples of starry flounder (*Plati-*

chthys stellatus) captured at sites in the San Francisco Bay area, the highest arithmetic mean of micronucleated erythrocytes was only 2.4 per thousand erythrocytes. These lesions were presumably comparable to the "M-B lesion combination" in the present paper. Similarly, Hose et al. (1987) determined the arithmetic mean of micronucleated erythrocytes among the white croaker collected from a contaminated site to be only 3.4 per mille and from an uncontaminated site to be 0.8 per mille. The low micronucleus frequencies observed by Metcalfe (1988) prompted him to conclude that the technique would not be suitable as an in vivo bioassay, and it was for this reason that fish were abandoned by Jaylet (1986a, 1986b) in favor of a pleurodele when exposure of two fish species to both X-irradiation and a known chemical clastogen resulted in unacceptably low micronucleus frequencies.

Second, the micronucleus test in fish may not be a sensitive measure of exposure to specific genotoxic chemicals. Even the well-established mammalian micronucleus test has been found to vary substantially in sensitivity because of the species, strain, and gender of the experimental animal (Styles et al. 1983); the approach and the statistical/technical protocol of the study (Heddle et al. 1983); and the organotropism which may or may not include a site of hematopoiesis (Trzos et al. 1978; Sobels 1987). It is for these reasons that mammalian cytogeneticists rely on the mouse micronucleus test only to indicate the presence, but not necessarily an absence, of genotoxic chemicals, even under the rigorous laboratory conditions considered essential for this technique (Trzos et al. 1978; Heddle et al. 1983; MacGregor et al. 1987).

The problems of low micronucleus frequencies and a lack of sensitivity were also encountered during the development of an in situ mammalian micronucleus bioassay intended to provide predictive tests of chemical carcinogenicity with the use of nucleated human and Chinese hamster ovary cells (Stich et al. 1983; Stich and Rosin 1983a, 1983b; Stich and Rosin 1984). For example, micronucleus frequencies in exfoliated human buccal cells were compared by Stich and Rosin (1983a) from several groups of humans known to orally ingest identified carcinogenic chemicals. In spite of the direct application of the genotoxic substances, however, the technique yielded significant differences from the control group only when the most synergistic carcinogenic agents were applied together.

In summary, we found the piscine micronucleus test to be of highly questionable value as an in situ indicator of biological effects in wild fish exposed to the chemical contaminants we measured. No definitive or consistent correlation was found between any observed variation of nuclear morphology and any measured level of chemical contamination in the sediments or in the white croaker bile or liver, nor with the prevalence of idiopathic liver lesions in the same specimens. Even if a relationship had been found, the frequencies of the observed lesions were extremely low and variable indicating that very large sample sizes would be required should this technique be found at some future date to actually have a dose-response relationship to chemical contaminants. Furthermore, we suspect that the erythrocyte nuclear lesions observed during this study may not have originated from a genotoxic event as a result of exposure to xenobiotic contaminants.

Acknowledgments

We thank Dr. Peggy Krahn and Don Brown of the National Analytical Facility, Division of Environmental Conservation, National Marine Fisheries Service, in Seattle, Washington, for the analyses of bile FACs and for the analyses of the white croaker liver and sediment contaminants, respectively, and for their review of this manuscript; Dr. Bruce McCain, Dr. Ed Casillas, and Lyndal Johnson for their review of the manuscript; Patricia Emry, Lyndal Johnson, and O. Paul Olson for the collection and preparation of the blood smears; Tom Lee for his histological preparation of the smears; and Ruth Mandapat for the age analysis of the white croaker otoliths. The collection of white croaker and sediments was performed as part of the West Coast portion of the National Benthic Surveillance Program conducted by the National Marine Fisheries Service. This work was funded in part by the Office of Oceanography and Marine Assessment, National Ocean Survey, of the U.S. National Oceanic and Atmospheric Administration (NOAA).

References

AL-SABTI, K. 1986a. Clastogenic effects of five carcinogenic-mutagenic chemicals on the cells of the common carp, *Cyprinus carpio* L. Comp. Biochem. Physiol. 85C(1): 5–9.

1986b. Comparative micronucleated erythrocyte cell induction in three cyprinids by five carcinogenic-mutagenic chemicals. Cytobios 47(190–191): 147–154.

AMOS, K. H. 1985. Procedures for the detection and identification of certain fish pathogens. 3rd ed. Fish Health Section, Am. Fish. Soc., Corvallis, OR. 114 p.

APPY, R. G., M. D. B. BURT, AND T. J. MORRIS. 1976. Viral nature of piscine erythrocytic necrosis (PEN) in the blood of Atlantic cod (*Gadus morhua*). J. Fish. Res. Board Can. 33: 1380–1385.

AMPHLETT, G. E., AND G. F. DELOW. 1984. Statistical analysis of the micronucleus test. Mutat. Res. 128: 161–166.

BESSIS, M. 1973. Living blood cells and their ultrastructure. Springer–Verlag, New York, NY. 767 p.

BEUTLER, E. 1985. Chemical toxicity of the erythrocyte, p. 39–49. *In* R. D. Irons [ed.] Toxicology of the blood and bone marrow. Raven Press, New York, NY. 182 p.

BRUNETTI, R., F. MAJONE, I. GOLA, AND C. BELTRAME. 1988. The micronucleus test: examples of application to marine ecology. Mar. Ecol. Prog. Ser. 44: 65–68.

CASTOLDI, G. L. 1981. Erythrocytes, p. 33–145. *In* D. Zucker-Franklin, M. F. Greaves, C. E. Grossi, and A. M. Marmont [ed.] Atlas of blood cells: function and pathology. Edi. Ermes, Milano, Italy.

CHEN, S. N., G. H. KOU, R. P. HEDRICK, AND J. L. FRYER. 1985. The occurrence of viral infections of fish in Taiwan, p. 313–319. *In* A. E. Ellis [ed.] Fish and shellfish pathology. Academic Press, London, UK.

DAS, R. K., AND N. K. NANDA. 1986. Induction of micronuclei in peripheral erythrocytes of fish *Heteropneustes fossilis* by mitomycin C and paper mill effluent. Mutat. Res. 175(2): 67–71.

ELLIOTT, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biol. Assoc. Sci. Publ. 25. Cumbria, UK. 156 p.

EVELYN, T. P. T., AND G. S. TRAXLER. 1978. Viral erythrocytic necrosis: natural occurrence in Pacific salmon and experimental transmission. J. Fish. Res. Board Can. 35: 903–907.

FORBES, D. J., M. W. KIRSCHNER, AND J. W. NEWPORT. 1983. Spontaneous formation of nucleus-like structures around bacteriophage DNA microinjected into Xenopus eggs. Cell 34: 13–23.

GHADIALLY, F. N. 1982. Ultrastructural pathology of the cell and matrix. 2nd ed. Butterworths, London, UK. 971 p.

GRAY, P. 1954. The microtomist's formulary and guide. McGraw–Hill, New York, NY.

GRINFELD, S., A. JAYLET, R. SIBOULET, P. DEPARIS, AND I. CHOUROULINKOV. 1986. Micronuclei in red blood cells of the newt *Pleurodeles waltl* after treatment with benzo[a]pyrene: dependence on dose, length of exposure, posttreatment time, and uptake of the drug. Environ. Muta. 8: 41–51.

HAYHOE, F. G. J., AND R. J. FLEMANS. 1982. Color atlas of hematological cytology. 2nd ed. John Wiley and Sons, New York, NY. 240 p.

HEDDLE, J. A., M. HITE, B. KIRKHART, K. MAVOURNIN, J. T. MACGREGOR, G. W. NEWELL, AND M. F. SALAMONE. 1983. The induction of micronuclei as a measure of genotoxicity, a report of the U.S. Environmental Protection Agency Gene-Tox program. Mutat. Res. 123: 61–118.

HENDRICKS, J. D., T. R. MEYERS, D. W. SHELTON, J. L. CASTEEL, AND G. S. BAILEY. 1985. Hepatocarcinogenicity of benzo[a]pyrene to rainbow trout by dietary exposure and intraperitoneal injection. J. Natl. Cancer Inst. 74(4): 839–851.

HOOFTMAN, R. N. 1981. The induction of chromosome aberrations in *Notobranchius rachowi* (Pisces: Cyprinodontidae) after treatment with ethyl methanesulphonate or benzo[a]pyrene. Mutat. Res. 91: 347–352.

HOOFTMAN, R. N., AND W. K. DE RAAT. 1982. Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mudminnow *Umbra pygmaea* by ethyl methanesulphonate. Mutuat. Res. 104: 147–152.

HOOFTMAN, R. N., AND G. J. VINK. 1981. Cytogenetic effects on the eastern mudminnow. *Umbra pygmaea*, exposed to ethyl methanesulfonate, benzo[a]pyrene, and river water. Ecotoxicol. Environ. Saf. 5: 261–269.

HOSE, J. E., J. N. CROSS, S. G. SMITH, AND D. DIEHL. 1987. Elevated circulating erythrocyte micronuclei in fishes from contaminated sites off southern California. Mar. Environ. Res. 22: 167–176.

HYUN, B. H., G. L. GULATI, AND J. K. ASHTON. 1986. Color atlas of clinical hematology. Igaku–Shoin, New York, NY. 272 p.

JANDL, J. H. 1987. Blood: textbook of hematology. Little, Brown and Co., Boston, MA. 1214 p.

JAYLET, A., P. DEPARIS, V. FERRIER, S. GRINFELD, AND R. SIBOULET. 1986a. A new micronucleus test using peripheral blood erythrocytes of the newt *Pleurodeles waltl* to detect mutagens in fresh-water pollution. Mutat. Res. 164: 245–257.

JAYLET, A., P. DEPARIS, AND D. GASCHIGNARD. 1986b. Induction of micronuclei in peripheral erythrocytes of axolotl larvae following *in vivo* exposure to mutagenic agents. Mutagenesis 1(3): 211–215.

KIMURA, D. K., R. R. MANDAPAT, AND S. L. OXFORD. 1979. Method, validity, and variability in the age determination of yellowtail rockfish (*Sebastes flavidus*), using otoliths, J. Fish. Res. Board Can. 36(4): 377–383.

KRAHN, M. M., D. G. BURROWS, W. D. MACLEOD, JR., AND D. C. MALINS. 1987. Determination of individual metabolites of aromatic compounds in hydrolyzed bile of English sole (*Parophrys vetulus*) from polluted sites in Puget Sound, Washington. Arch. Environ. Contam. Toxicol. 16: 511–522.

KRAHN, M. M., M. S. MYERS, D. G. BURROWS, AND D. C. MALINS. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. Xenobiotica 14(8): 633–646.

KRAHN, M. M., L. D. RHODES, M. S. MYERS, L. K. MOORE, W. D. MACLEOD, JR., AND D. C. MALINS. 1986. Associations between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Arch. Environ. Contam. Toxicol. 15: 61–67.

KRAHN, M. M., C. A. WIGREN, R. W. PEARCE, L. K. MOORE, R. G. BOGAR, W. D. MACLEOD, JR., S.-L. CHAN, AND D. W. BROWN. 1988. Standard analytical procedures of the NOAA National Analytical Facility, 1988: new HPLC cleanup and revised extraction procedures for organic contaminants. Nat'l Oceanic Atmos. Adm. Tech. Memo. NMFS F/NWC-153, 52 p.

LANDOLT, M. L., AND R. M. KOCAN. 1983. Fish cell cytogenetics: a measure of the genotoxic effects of environmental pollutants, p. 335–353. *In* J. O. Nriagu [ed.] Aquatic toxicology. John Wiley and Sons, New York, NY.

LEEK, S. L. 1987. Viral erythrocytic inclusion body syndrome (EIBS) occurring in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) reared in freshwater. Can. J. Fish. Aquat. Sci. 44: 685–688.

LOHKA, M. J., AND Y. MASUI. 1984. Roles of cytosol and cytoplasmic particles in nuclear envelope assembly and sperm pronuclear formation in cell-free preparations from amphibian eggs. J. Cell Biol. 98: 1222–1230.

LONG, E. R., AND M. F. BUCHMAN. 1989. An evaluation of candidate measures of biological effects for the National Status and Trends Program. Nat'l Oceanic Atmos. Adm. Tech. Memo. NOS OMA-45, 97 p.

LONGWELL, A. CROSBY, D. PERRY, J. B. HUGHES, AND A. HEBERT. 1983. Frequencies of micronuclei in mature and immature erythrocytes of fish as an estimate of chromosome mutation rates — results of field surveys on windowpane flounder, winter flounder, and Atlantic mackeral. Int. Counc. Explor. Sea, C.M. 1983/E:55.

LOVE, M. S., G. E. MCGOWEN, W. WESTPHAL, R. J. LAVENBERG, AND L. MARTIN. 1984. Aspects of the life history and fishery of the white croaker, *Genyonemus lineatus* (Sciaenidae), off California. U.S. Nat. Mar. Fish. Serv. Fish. Bull. 82(1): 179–198.

MACGREGOR, J. T., J. A. HEDDLE, M. HITE, B. H. MARGOLIN, C. RAMEL, M. F. SALAMONE, R. R. TICE, AND D. WILD. 1987. Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. Mutat. Res. 189: 103–112.

MACLEOD, W. D., JR., D. W. BROWN, A. J. FRIEDMAN, D. G. BURROWS, O. MAYNES, R. W. PEARCE, C. A. WIGREN, AND R. G. BOGAR. 1985. Standard anaytical procedures of the NOAA National Analytical Facility, 1985–1986: extractable toxic organic compounds. 2nd ed. Nat'l Oceanic Atmos. Adm. Tech. Memo. NMFS F/NWC-92, 121 p.

MALINS, D. C., M. M. KRAHN, M. S. MYERS, L. D. RHODES, D. W. BROWN, C. A. KRONE, B. B. MCCAIN, AND S.-L. CHAN. 1985. Toxic chemicals in sediments and biota from a creosote-polluted harbor: relationships with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). Carcinogenesis (Lond.) 6(10): 1463–1469.

MALINS, D. C., B. B. MCCAIN, D. W. BROWN, M. S. MYERS, M. M. KRAHN, AND S.-L. CHAN. 1987a. Toxic chemicals, including aromatic and chlorinated hydrocarbons and their derivatives, and liver lesions in white croaker (*Genyonemus lineatus*) from the vicinity of Los Angeles. Environ. Sci. Technol. 21(8): 765–770.

MALINS, D. C., B. B. MCCAIN, M. S. MYERS, D. W. BROWN, M. M. KRAHN, W. T. ROUBAL, M. H. SCHIEWE, J. T. LANDAHL, AND S.-L. CHAN. 1987b. Field and laboratory studies of the etiology of liver neoplasms in marine fish from Puget Sound. Environ. Health Perspect. 71: 5–16.

MANNA, G. K., G. BANERJEE, AND S. GUPTA. 1985. Micronucleus test in the peripheral erythrocytes of the exotic fish, *Oreochromis mossambica*. Nucleus (Calcutta) 28(3): 176–179.

METCALFE, C. D. 1988. Induction of micronuclei and nuclear abnormalities in the erythrocytes of mudminnows (*Umbra limi*) and brown bullheads (*Ictalurus nebulosus*). Bull. Environ. Contam. Toxicol. 40: 489–495.

MILLER, R. G., JR. 1981. Simultaneous statistical inference. 2nd ed. Springer–Verlag, New York, NY. 299 p.

MYERS, M. S., L. D. RHODES, AND B. B. MCCAIN. 1987. Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions, and other idiopathic hepatic conditions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. J. Natl. Cancer Inst. 78(2): 333–363.

NEWMAN, M. 1985. Viral Erythrocytic Necrosis. Fiches Identif. Mal. Parasit. Poissons Crust. Mollusques, leaflet no. 22. Cons. Int. Explor. Mer, Copenhagen, Denmark. 5 p.

OSBORNE, M. R., AND N. T. CROSBY. 1987. Benzopyrenes. Cambridge University Press, Cambridge, UK. 329 p.

PLATT, W. R. 1979. Color atlas and textbook of hematology. 2nd ed. J. B. Lippincott Co., Philadelphia, PA. 644 p.

POST, G. 1987. Textbook of fish health. Rev. ed. T.F.H. Publications, Neptune City, NJ. 288 p.

PREIN, A. E., G. M. THIE, G. M. ALINK, J. H. KOEMAN, AND C. L. M. POELS. 1978. Cytogenetic changes in fish exposed to water of the river Rhine. Sci. Total Environ. 9: 287–291.

RENO, P. W., M. PHILIPPON–FRIED, B. L. NICHOLSON, AND S. W. SHERBURNE. 1978. Ultrastructural studies of piscine erythrocytic necrosis (PEN) in Atlantic herring (*Clupea harengus harengus*). J. Fish. Res. Board Can. 35: 148–154.

RICKER, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

ROHOVEC, J. S., AND A. AMANDI. 1981. Incidence of viral erythrocytic necrosis among hatchery reared salmonids of Oregon. Fish Pathol. 15(3/4): 135–141.

SCHLEGEL, R., J. T. MACGREGOR, AND R. B. EVERSON. 1986. Assessment of cytogenetic damage by quantitation of micronuclei in human peripheral blood erythrocytes. Cancer Res. 46: 3717–3721.

SCHMID, W. 1975. The micronucleus test. Mutat. Res. 31: 9–15.

1976. The micronucleus test for cytogenetic analysis, p. 31–53. *In* A. Hollaender [ed.] Chemical mutagens: principles and methods for their detection. Vol. 4. Plenum Press, New York, NY.

SHERBURNE, S. W. 1973. Erythrocyte degeneration in the Atlantic herring, *Clupea harengus harengus* L. U. S. Nat. Mar. Fish. Serv. Fish. Bull. 71(1): 125–134.

SIBOULET, R., S. GRINFIELD, P. DEPARIS, AND A. JAYLET. 1984. Micronuclei in red blood cells of the newt *Pleurodeles waltl* Michah: induction with X-rays and chemicals. Mutat. Res. 125: 275–281.

SMAIL, D. A. 1982. Viral erythrocytic necrosis in fish: a review. Proc. R. Soc. Edinb. 81B: 169–176.

SMAIL, D. A., AND S. I. EGGLESTONE. 1980. Virus infections of marine fish erythrocytes: prevalence of piscine erythrocytic necrosis in cod *Gadus morhua* L. and blenny *Blennius pholis* L. in coastal and offshore waters of the United Kingdom. J. Fish. Dis. 3: 41–46.

SMITH, C. E. 1968. Hematological changes in coho salmon fed a folic acid deficient diet. J. Fish. Res. Board Canada 25(1): 151–156.

SMITH, C. E., AND J. E. HALVER. 1969. Folic acid anemia in coho salmon. J. Fish. Res. Board Canada 26(1): 111–114.

SOBELS, F. H. 1987. In vitro testing as a step in the evaluation of in vivo genotoxicity. Mutat. Res. 189: 7–10.

SOKAL, R., AND F. ROHLF. 1969. Biometry. W. F. Freeman, San Francisco, CA. 859 p.

SPIES, R. B., J. STEGEMAN, P. THOMAS, D. W. RICE, JR., J. CROSS, AND M. PRIETO. 1987. Organic contaminant concentrations, microsomal P-450

catalytic activity, plasma sex steroids, erythrocyte micronuclei formation, and reproductive success in *Platichthys stellatus* from San Francisco Bay. Presented at Western Society of Naturalists 68th Annual Meeting, Dec. 26–30 1987, at Calif. State Univ., Long Beach. (Abstract only).

STICH, H. F., B. A. BOHM, K. CHATTERJEE, J. L. SAILO. 1983. The role of saliva-borne mutagens and carcinogens in the etiology of oral and esophageal carcinomas of betel nut and tobacco chewers, P. 43–58. *In* H. F. Stich [ed.] Carcinogens and mutagens in the environment. Vol. III. Naturally occurring compounds. CRC Press, Boca Raton, FL.

STICH, H. F., AND M. P. ROSIN. 1983a. Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells. Int. J. Cancer 31: 305–308.

1983b. Micronuclei in exfoliated human cells as an internal dosimeter for exposures to carcinogens, p. 17–25. *In* H. F. Stich [ed.] Carcinogens and mutagens in the environment. Vol. II. Naturally occurring compounds: endogenous formation and modulation. CRC Press, Boca Raton, FL.

1984. Micronuclei in exfoliated human cells as a tool for studies in cancer risk and cancer intervention. Cancer Lett. 22: 241–253.

STYLES, J. A., C. R. RICHARDSON, AND B. BURLINSON. 1983. A comparison of the incidence of micronuclei in blood and bone marrow in three strains of mouse dosed with cyclophosphamide or hexamethylphosphoramide (HMPA). Mutat. Res. 122: 143–147.

THILLY, W. G., AND K. M. CALL. 1986. Genetic toxicology, p. 174–194. *In* C. D. Klaassen, M. O. Amdur, and J. Doull [ed.] Casarett and Doull's toxicology: the basic science of poisons. 3rd ed. Macmillan Publishing Company, New York, NY.

TICE, R. R., AND J. L. IVETT. 1985. Cytogenetic analysis of bone marrow damage, p. 119–140. *In* R. D. Irons [ed.] Toxicology of the blood and bone marrow. Raven Press, New York, NY. 182 p.

TRZOS, R. J., G. L. PETZOLD, M. N. BRUNDEN, AND J. A. SWENBERG. 1978. The evaluation of sixteen carcinogens in the rat using the micronucleus test. Mutat. Res. 58: 79–86.

VARANASI, U., M. NISHIMOTO, W. L. REICHERT, AND B.-T. L. EBERHART. 1986. Comparative metabolism of benzo[*a*]pyrene and covalent binding to hepatic DNA in English sole, starry flounder, and rat. 1986. Cancer Res. 46: 3817–3824.

VARANASI, U., S.-L. CHAN, B. B. MCCAIN, J. T. LANDAHL, M. H. SCHIEWE, R. C. CLARK, D. W. BROWN, M. S. MYERS, M. M. KRAHN, W. D. GRONLUND, AND W. D. MACLEOD, JR. 1989a. National Benthic Surveillance Project: Pacific coast. Part II, technical presentation of the results for cycles I to III (1984-86). NOAA Tech. Memo. NMFS F/NWC-170, 158 p.

VARANASI, U., J. E. STEIN, AND M. NISHIMOTO. 1989b. Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAH) in fish, p. 93–149. *In* U. Varanasi [ed.] Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, FL. 341 p.

ZAR, J. H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, NJ. 718 p.

Factors Influencing Fish Assemblages and Species Richness in Subtropical Florida Lakes and a Comparison with Temperate Lakes

A. E. Keller[1] and T. L. Crisman

Department of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611, USA

Keller, A. E., and T. L. Crisman. 1990. Factors influencing fish assemblages and species richness in subtropical Florida lakes and a comparison with temperate lakes. Can. J. Fish. Aquat. Sci. 47: 2137–2146.

From detailed analyses of 36 lakes, we determined that while species assemblages and richness change with lake size, trophic state, and pH, the responses were not as dramatic in Florida as in temperate lakes. At a given pH, there were more species in Florida lakes than in comparable lakes of the temperate zone. The impact of lake size on species richness was also less obvious in Florida lakes. The greater importance of centrarchids, increased contribution of the littoral zone to primary production, and lower dissolved aluminum levels may contribute to greater species richness in Florida lakes than in temperate lakes of comparable pH or size.

L'analyse détaillée de trente-six (36) lacs a permis de constater que même si l'abondance et les mélanges d'espèces varient suivant les dimensions, le niveau trophique et le pH du lac, les réactions n'ont pas été aussi marquées en Floride que dans les lacs de la zone tempérée. À un pH donné, il y avait un plus grand nombre d'espèces dans les lacs de la Floride que dans des lacs comparables de la zone tempérée. L'effet des dimensions du lac sur l'abondance des espèces était également moins évident dans les lacs de la Floride. La plus grande importance des centrarchidés, la contribution accrue de la zone littorale dans la production primaire et les niveaux moins élevés d'aluminium dissous peuvent contribuer à une pus grande abondance des espèces dans les lacs de Floride que dans les lacs de dimensions et de pH comparables situés dans la zone tempérée.

Received June 26, 1988
Accepted May 10, 1990
(J9792)

Reçu le 26 juin 1988
Accepté le 10 mai 1990

Loss of fish from acidified lakes has been attributed to heavy metal and aluminum toxicity (Baker 1982; Wright et al. 1981; Norton and Davis 1981), loss of prey species (Ryan and Harvey 1980), recruitment failure (Jensen and Snekvik 1972; Ryan and Harvey 1977) and direct pH effects on fish physiology (Fromm 1981; Wood and McDonald 1982). Field investigations of fish communities in Scandinavia (Wright and Snekvik 1978; Almer et al. 1974), eastern Canada (Harvey and Lee 1982; Kelso and Minns 1982) and northern United States (Schofield 1976; Rahel 1983; Weiner et al. 1984; Tonn and Magnuson 1982) indicated that the pH was significantly related to number of fish species. While many salmonids and cyprinids prefer relatively alkaline waters (Rahel 1983; Harvey and Lee 1982), other taxa have adapted to acidic conditions and/or may be restricted to such environments (Hastings 1979; Rahel 1984; Jewell and Brown 1929; Dunson et al. 1977).

Acid deposition is not restricted to temperate zones. Although approximately half as acidic as that of the Midwest and Northeast, mean annual precipitation pH in subtropical Florida averaged 4.68 in 1988 (Florida Acid Deposition Monitoring Program 1989). Approximately 31% of Florida's 7700 lakes are considered sensitive to acidic deposition (Canfield et al. 1983), and a recent United States Environmental Protection Agency (USEPA) national survey documented that Florida possesses both the greatest number of acidic lakes and the lakes of lowest pH of any region in the eastern United States (Linthurst et al. 1986). While pH appears to have exhibited long-term stability in most lakes in the peninsula, it appears that some lakes in northern Florida have undergone recent acidification (Sweets et al. 1989; Charles et al. 1986).

Given the broader pH range, relative temporal stability of acidity, and decreased importance of covarying factors influencing biotic responses (aluminum, loss of predators), Florida lakes can provide valuable insight into the relation of fish communities with environmental attributes, including pH. In this study we have: (1) identified fish species assemblages and factors structuring them in 36 subtropical Florida lakes, (2) examined the relationship between fish species richness and both pH and lake size for these same lakes, and (3) compared our results to data for lakes in three north temperate regions — the La Cloche Mountains (Harvey 1975), the Adirondack Mountains (Adirondack Lake Survey Corp. 1989), and northern Wisconsin (Rahel 1984).

Methods

Fish Assemblages

Water chemistry and fish species lists were compiled for 36 Florida lakes from surveys conducted between 1975 and 1983. Water chemistry data (Appendix A) represent annual means of three to four evenly spaced sampling events (Canfield 1981; Garren et al. 1984; Hendry 1977). Chemical analyses were performed using standard methods (American Public Health Association 1981; United States Environmental Protection Agency 1979). Lake surface area, mean and maximum depth, and volume were calculated from current bathymetric maps of

[1]Author to whom correspondence should be addressed. Current address: Tennessee Valley Authority — Aquatic Biology Department, HB 2S 270C-C, 311 Broad Street, Chattanooga, TN 37402-2801, USA.

the United States Geological Survey (Kenner 1964), the Florida Game and Fresh Water Fish Commission (1978) and Garren et al. (1984).

Fish species composition were based on presence-absence counts from the database of the Florida Game and Fresh Water Fish Commission. In addition to a single sampling with rotenone, each lake was sampled by one or more additional methods, e.g. gill nets, trammel nets, electroshocking. All data on introduced species such as *Tilapia aurea* and *Dorosoma petenense* were excluded from our data set. Physico-chemical data (except pH) and species counts were normalized via a $Log_{10} + 1$ transformation.

The SAS (SAS 1982) Varclus procedure was used to identify species assemblages. Varclus is an iterative clustering technique designed to divide a set of variables into non-overlapping groups based on the first principal component. Since we were interested in identifying species associations, the input data were species lists for each of the 36 lakes. A plot of cubic clustering criterion vs. number of clusters (Sarle 1983) and subsequent examination of eigenvalues indicated that four clusters of fish species optimized the proportion of variance (45%) explained by the fewest number of clusters.

The biological validity of the four fish clusters was tested by first assigning the lakes to four lake groups based on fish cluster loadings for each lake produced by the SAS SCORE procedure. A fifth group consisted of nine lakes that were not strongly associated with any fish cluster. Next, chemical and physical parameters for the 36 lakes were analyzed by ANOVA and Duncan's multiple range test to identify characteristics that were significantly different among the five lake groups. Finally, we considered the importance of biogeography, lake location, and fish habitat preferences on observed fish fauna of each lake group.

Relationships Between Species Richness and Physico-Chemical Parameters

Correlation analysis was used to identify which physico-chemical characteristics were related to fish species richness for the 36 Florida lakes. Because virtually all of the parameters examined were significantly correlated with species richness, partial correlations were extracted (Nie 1975). Next, using results of the partial correlation analysis, multiple linear regression equations were produced using the SAS Stepwise procedure with 0.15 as the significance level for entry of parameters.

Interregional Comparisons

Fish species and water chemistry data from 138 northern Wisconsin lakes (Rahel 1982), 68 La Cloche Mountain lakes (Harvey 1975) and 50 lakes from the Adirondack Mountains (Adirondack Lake Survey Corp. 1989) were compared to data from our 36 lakes. Analysis of covariance was used to determine whether apparent regional differences in the species richness–lake size and species richness–lake pH regressions were significant. All statistical analyses were performed with SAS (1982) procedures at the North East Regional Data Center, University of Florida.

Results

Species Assemblages

Forty-eight fish species were identified from the 36 Florida lakes (Table 1) and were separated into four clusters. Largemouth bass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*) were ubiquitous and therefore were not part of any cluster. The lakes, ranging in size from 0.065–129.6 km^2, and mean annual chlorophyll *a* and pH of 0.55–145 μg/L and 4.1–8.9, respectively (Table 2), were assigned to four groups based on the strength of their association with individual fish clusters. A fifth group consisted of the nine lakes lacking strong loadings with any fish cluster.

Nine of the ten fish species comprising Cluster I were euryhaline species (Myers 1938) including Atlantic needlefish (*Anguilla rostrata*), sheepshead minnow (*Cyprinodon variegatus*), tidewater silverside (*Menidia beryllina*) and several killifish taxa. These fish would be expected to populate lakes with marine connectiions (C. R. Gilbert, Florida Museum of Natural History, Gainesville, FL, pers. comm.; Lee et al. 1980). ANOVA and Duncan's test indicated that Group 1 lakes (strongly related to Cluster I fish) were significantly different from the other lake groups (Table 3) and are characterized by high chlorophyll *a* (mean = 112.20 μg/L), algal domination (mean % weed cover = 2.10) and large surface area (mean = 43.38 km^2). Lakes Apopka, Dora, and Griffin, virtually the only lakes with Cluster I fish (Table 1), have a major marine connection via the Oklawaha and St. Johns Rivers. Thus, euryhaline species have both an immigration route into these lakes, and conditions favorable for survival (mean conductivity = 309.03 μmhos/cm, alkalinity = 114.81 mg/L as $CaCO_3$, pH = 8.8).

The 12 fish species in Cluster II are likely to be found in weedy habitats (Lee et al. 1980) with many species preferring acidic wetlands (Hastings 1979; Gilbert 1987). Redfin pickerel (*Esox americanus*), spotted gar (*Lepisosteus oculatus*) and several small sunfish are indicative of this group. The Group 2 lakes characterized by Cluster II fish have been plagued by problems with submergent macrophytes (44.25% weed cover) (Florida Game and Fresh Water Fish Commission 1960 and 1978; Huber et al. 1982), have moderate chlorophyll *a* levels (mean = 7.24 μg/L), are softwater (mean alkalinity = 2.82 mg/L as $CaCO_3$), slightly colored (64.56 Pt-Co units), and acidic (pH = 5.06).

Cluster III contained ten species with warmouth sunfish (*Lepomis gulosus*), brook silverside (*Labidesthes sicculus*), chain pickerel (*Esox niger*), and yellow bullhead (*Ictalurus natalis*) being the most important. These fish typically inhabit drainage lakes that are less weedy than those in Cluster II (Lee et al. 1980). Twelve of the 13 lakes most strongly characterized by this fish cluster are located in the Florida panhandle, the most temperate region of the state. Thus, along with being clear water (mean color = 9.55 Pt-Co units), these lakes are moderately weeded (% weed cover = 23.67) and oligo-mesotrophic (chlorophyll *a* = 4.17 μg/L).

Cluster IV fish (e.g. Florida gar, gizzard shad, black crappie, and white catfish) are found in open water habitats of productive, large lakes. Four of the eight largest lakes in the data set (Sante Fe, Sampson, Eaton, and Lochloosa) were either dominated by Cluster IV species or contained a high percentage (≥50%) of such fish. The smaller lakes (0.71–1.2 km^2) that also had these open water species (Chain O'Lakes and Eaton) are connected to major rivers (St. Johns and Oklawaha Rivers, respectively). Group 4 lakes, i.e. those with high loadings on fish Cluster IV, were large (mean surface area = 3.14 km^2), moderately productive (mean chlorophyll *a* = 5.25 μg/L), and slightly colored (64.56 Pt-Co units). These lakes also had the second highest pH (6.10) of the four groups. Three large lakes

TABLE 1. Fish species clusters vs. lake groups matrix. Lake numbers correspond to Appendi

Species	Number	Group 1			Group 2												G
		3	10	14	4	1	19	16	25	35	17	11	24	8	18		
Atlantic needlefish		X		X													
Least killifish		X		X													
Pugnose minnow				X													
Bluefin killifish			X	X			X										
Redbreast sunfish				X													
Sailfin mollie				X													
Seminole killifish		X	X	X													
Sheepshead minnow				X													
Striped bass				X													
Tidewater silverside		X		X													
Blue-spotted sunfish		X				X	X	X	X	X	X						
Redfin pickerel					X	X			X		X						X
Spotted gear						X		X	X					X			
Swamp darter			X	X		X	X	X	X			X					X
Starhead topminnow					X		X	X	X			X					
Pirate perch						X		X									
Banded pygmy sunfish									X								
Spotted sunfish				X	X				X								X
Round flier								X				X					
Black-banded sunfish																	
Red minnow									X								
Banded topminnow																	
Brook silverside			X			X	X		X			X		X			
Chain pickerel				X			X	X	X	X		X		X			
Channel catfish			X		X								X				
Lake chubsucker		X		X	X	X	X	X	X	X	X	X	X	X			X
Dollar sunfish			X	X		X				X	X	X		X			X
Pygmy killifish												X					
Brown darter																	
Warmouth sunfish		X	X	X	X	X	X	X	X	X	X	X	X	X			X
Yellow bullhead		X	X	X	X	X			X	X	X	X		X			X
Everglades pygmy sunfish												X					
Banded sunfish																	
American eel		X		X					X								
Black crappie		X	X	X		X	X	X	X	X	X	X	X				
Bowfin		X	X	X		X			X	X	X		X				
Brown bullhead		X	X	X		X	X		X	X							
Florida gar		X	X	X			X			X							
Mosquitofish		X	X	X				X	X	X	X		X				
Gizzard shad		X	X	X		X											
Golden shiner		X	X	X		X	X	X	X								
Long-nosed gar		X	X	X		X			X								
Redear sunfish		X	X	X	X	X	X		X	X	X		X				X
Tadpole madtom		X	X	X		X			X			X					X
Taillight shiner		X	X	X					X								
White catfish		X	X														
Golden topminnow					X		X	X									X
Largemouth bass		X	X	X	X	X	X	X	X	X	X	X	X	X			X
Bluegill sunfish		X	X	X	X	X	X	X	X	X	X	X	X	X			X

Appendix A.

Group 3										Group 4						Group 5								
4	8	18	34	13	15	33	28	29	27	26	31	12	20	30	7	22	23	9	21	32	6	5	2	36
										X														
												X												
														X										
				X	X						X		X		X					X		X	X	
		X	X		X	X		X																
	X			X			X																	
		X		X			X		X			X	X	X		X	X		X		X			
				X																	X			
		X			X															X				
															X					X				
	X			X		X	X		X	X	X	X	X	X		X	X		X	X	X	X	X	
	X			X			X	X	X	X	X		X						X	X				
					X										X									
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X			X			X
	X	X	X	X	X	X	X	X	X	X					X							X		X
		X		X																				
				X																				
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X
	X	X	X	X	X	X	X	X	X	X					X							X		X
				X																				
							X																	
												X												
			X	X				X	X	X		X	X	X						X				
							X			X	X	X	X		X									
								X	X		X	X	X											
			X	X						X		X	X		X	X	X							
											X	X	X	X										
										X	X	X	X	X	X	X				X		X		
		X	X		X	X		X	X	X	X	X	X	X	X	X			X	X				
		X		X	X	X			X	X	X	X	X	X										
											X	X	X	X		X								
											X		X											
		X			X	X		X							X					X	X			
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

TABLE 2. Means (ranges) of chemical and physical characteristics of the 36 study lakes divided into groups based on fish assemblages. Group 5 represents a set of lakes that were not significantly different for a parameter based on Duncan's multiple range test ($P \leq 0.05$).

Parameter	Lake Group 1 ($n=3$)	Lake Group 2 ($n=5$)	Lake Group 3 ($n=13$)	Lake Group 4 ($n=6$)	Lake Group 5 ($n=9$)
pH	8.80[a]	5.06[bc]	5.47[bc]	6.10[b]	4.85[c]
	(8.63–8.91)	(4.08–5.99)	(4.54–7.72)	(4.84–7.37)	(4.59–5.19)
Ca	35.48[a]	2.14[c]	2.34[c]	5.75[b]	2.13[c]
	(30.90–39.81)	(1.29–3.47)	(1.10–18.62)	(1.70–12.89)	(1.66–6.31)
Na	17.78[a]	3.98[bc]	3.31[c]	6.76[b]	5.13[bc]
	(15.49–20.42)	(2.57–6.02)	(2.04–16.98)	(4.47–12.88)	(3.47–14.45)
Cl	30.90[a]	5.62[bc]	4.07[c]	10.0[b]	9.12[b]
	(24.55–36.31)	(3.98–10.47)	(1.41–19.05)	(8.51–11.48)	(5.37–25.70)
K	8.71[a]	1.23[b]	1.38[b]	1.41[b]	1.23[b]
	(5.75–10.96)	(1.10–1.62)	(1.17–3.80)	(1.35–2.24)	(1.10–1.58)
TN	4.17[a]	1.41[b]	1.38[b]	1.82[b]	1.48[b]
	(3.16–5.62)	(1.23–1.62)	(1.15–3.16)	(1.35–2.24)	(1.10–2.09)
Alkalinity	114.81[a]	2.82[b]	3.23[b]	6.92[b]	2.69[b]
	(97.72–131.82)	(0–3.98)	(0–58.88)	(3.02–23.99)	(1.51–6.16)
Surface area	43.38[a]	2.44[b]	1.13[bc]	3.14[b]	0.38[c]
	(18.08–128.02)	(0.44–23.29)	(0.13–4.87)	(0.29–23.08)	(0.07–5.10)
Chlorophyll	112.20[a]	7.24[b]	4.17[bc]	5.25[bc]	2.14[c]
	(74.13–158.49)	(4.26–11.75)	(1.55–144.54)	(1.90–33.11)	(1.70–3.02)
Secchi depth	1.32[a]	2.40[b]	3.98[c]	2.40[b]	3.98[c]
	(1.20–1.41)	(1.99–6.02)	(1.41–6.02)	(1.29–3.63)	(2.40–5.75)
Color	33.88[ab]	64.56[a]	9.55[b]	64.56[a]	16.60[ab]
	(23.99–43.65)	(30.20–194.98)	(0–52.48)	(12.88–616.60)	(3.98–46.86)
Conductivity	309.03[a]	27.54[c]	25.70[c]	61.66[c]	35.48[bc]
	(263.03–338.84)	(16.98–58.88)	(14.12–186.21)	(25.12–125.89)	(23.99–47.86)
Weed cover (%)	2.10[a]	44.25[b]	23.67[ab]	19.20[ab]	48.00[b]
	(0.3–4.0)	(9–100)	(5–65)	(1–49)	(1–100)
Number fish species	29	19.6	11	16	7.44
	(23–33)	(11–28)	(6–18)	(13–19)	(5–12)

All ionic concentrations in mg/L. TN is total nitrogen and is given in mg/L. Chlorophyll *a* concentrations are µg/L. Alkalinity is in mg/L as $CaCO_3$. Color is in mg/L as Pt. Conductivity is in µmhos/cm. Secchi depth is in metres. Surface area is in km^2.

TABLE 3. Simple and partial correlations for number of fish species vs. various chemical and physical characteristics for 36 Florida lakes.

Species vs	Simple correlation coefficient	Partial correlation coefficient controlling for pH	Partial correlation coefficient controlling for SA
pH	0.60[a]	—	0.32[a]
Na^+	0.41[a]	−0.003	0.07
Ca^{2+}	0.53[a]	−0.05	0.22
K^+	0.52[a]	0.02	0.27
Total nitrogen	0.46[a]	−0.05	0.16
Alkalinity	0.49[a]	−0.14	0.16
Secchi depth	−0.55[a]	−0.26	−0.27
Chlorophyll *a*	0.58[a]	0.20	0.26
Surface area	0.65[a]	0.53[b]	—

[a]Significant at $P \leq 0.05$
[b]Significant at $P \leq 0.01$. All others not significant.

with a marine connection (Apopka, Dora, and Griffin) that were assigned to Group 1 were also very strongly associated with Cluster IV (Table 1).

The fish assemblages of a number of lakes, e.g. McCloud, Magnolia, Lowry, Doe, and Buck, could not be assigned to any of the four fish clusters. ANOVA and Duncan's multiple range test helped to identify these lakes as a separate group (Group 5) based on water chemistry. Group 5 lakes are the most acidic (pH = 4.85), least productive (chlorophyll $a = 2.14$ µg/L), smallest (surface area = 0.38 km^2) and most insular lakes of the data base. All are located along the Trail Ridge of north-central Florida.

Species Richness

Lake size, pH, chlorophyll *a*, Secchi depth, total Kjeldhal nitrogen, Na^+, K^+, alkalinity, color, volume, conductivity, and Ca^{+2} were all significantly correlated ($P \leq 0.05$) with species richness (Table 3), while total phosphorus and depth were not. However, many Florida lakes lie on phosphate-rich marine deposits and are therefore nitrogen-limited (Canfield 1981). Lake depth did not vary greatly within our data set, the majority of lakes being 1–3 m deep.

Since virtually all of the lake parameters analyzed (96%) were correlated with each other as well as with species number, we used partial correlations and found that lake size was the most important characteristic related to fish species richness (Table 3). The only other characteristic of significance was pH; however, pH was not as important as lake size. Simple regression models relating the number of fish species to lake variables (Table 4) indicated that lake size was the best predictor. The best multiple regression model ($R^2 = 0.51$) produced by the SAS stepwise procedure included only surface area and pH, with the former accounting for 45% of the variance, while pH accounted for only an additional 6%. The importance of pH in the model may have been limited by its collinearity with surface area ($r = 0.54$).

TABLE 4. Results of regression analyses between number of fish species present in 36 Florida lakes and various lake characteristics. All regressions were statistically significant ($P \leq 0.05$).

Independent variate	Equation[a]	R^2
Lake size	$Y = 0.75 \text{ Log}_{10} (SA+1) + 1.78$	0.45
pH	$Y = 0.43 \text{ pH} + 1.27$	0.36
Chlorophyll *a*	$Y = 0.91 \text{ Log}_{10} (\text{Chla}+1) + 3.04$	0.34
Secchi depth	$Y = -2.32 \text{ Log}_{10} (SD+1) + 4.86$	0.30
Total nitrogen	$Y = 2.48 \text{ Log}_{10} (\text{TN}+1) + 3.19$	0.21
Ca^{2+}	$Y = 3.12 \text{ Log}_{10} (\text{Ca}+1) + 3.12$	0.29

[a]*SA* = surface area (km^2); Chla = Chlorophyll *a* (μg/L); *SD* = Secchi depth (m); TN = Total Kjeldhal nitrogen (mg/L); Ca = Calcium (mg/L).

Interregional Comparisons of Species Richness

Fish species richness and lake parameters for the 36 Florida lakes were compared by regression analysis to data for 138 lakes of northern Wisconsin representing a broad pH spectrum considered temporally stable (Rahel 1982), and lakes from two regions that have undergone anthropogenic acidification — the LaCloche Mountains of Ontario (Harvey 1975) and the Adirondack Mountains of New York (Adirondack Lake Survey Corp. 1989).

The Wisconsin data set included both dystrophic and clear lakes. The pH of these lakes ranged from 4.0–9.2, sizes were 0.004–15.66 km^2, and Secchi depths were from 0.8–8.7 m. The 68 La Cloche Mountain lakes ranged in pH from 3.8–7.4, had surface areas from 0.034–11.02 km^2 and Secchi depths from 2–14 m. La Cloche lakes have become progressively acidified during the last several decades (Harvey 1975, 1980). The 50 lakes of the Adirondack Mountain region ranged in pH from 4.4–8.9, in surface area from 0.0040–0.8340 km^2, and Secchi depths from 1.0–4.9 m. Several studies (Schofield 1977 and 1982; Pieffer and Festa 1980; Charles et al. 1986) have determined that many lakes in the Adirondack Mountains have acidified in recent years resulting in the loss of fish. Interregional comparisons of factors affecting fish species richness were limited to pH and surface area because of the availability of appropriate data.

There was a positive, significant ($P \leq 0.05$) relationship between number of fish species and lake pH in all four regions (Fig. 1). pH accounted for 33%, 35%, 50%, and 43% of the variance in the model for Florida, La Cloche Mountain lakes, Wisconsin, and Adirondack Mountain lakes, respectively. Analysis of covariance indicated that while there were no significant differences among regressions of species richness vs. pH for temperate lake regions, they were all significantly different from Florida. Therefore, data for Wisconsin, the La Cloche, and the Adirondack Mountains (Fig. 1) were pooled ("North"). The slope of the regression line relating species richness and pH was much greater for the "North" (0.24) than for Florida lakes (0.09).

The relationship between lake surface area and fish species richness was significant ($P \leq 0.05$) for lakes in all four regions — Wisconsin ($R^2 = 0.69$), La Cloche Mountains ($R^2 = 0.43$), Adirondack Mountains ($R^2 = 0.09$), and Florida ($R^2 = 0.43$) — but interregional comparisons were less conclusive than for pH (Fig. 2). There were significant differences ($P \leq 0.05$) in slopes between Florida (slope = 0.16) and all three temperate regions (Wisconsin slope = 0.37, Canadian slope = 0.36, and Adirondack slope = 0.22), and between the Adirondack Mountains and both La Cloche and Wisconsin. However, comparisons of slopes between Wisconsin and La Cloche Mountain lakes showed non-significance. Because intercepts for regression models of lake size and species richness were significantly different, data were not pooled.

Discussion

Species Assemblages

Fish assemblages in the Florida lakes were clustered along a gradient based on lake size, pH, color, dissolved solids, and partitioning of primary production into algal or macrophyte components. Consistent with observations made elsewhere (Rahel 1984; Hastings 1979; Tonn and Magnuson 1982), largemouth bass and bluegill were found in all lakes regardless of size, trophic state, color, or pH. Other widely distributed species included the lake chubsucker, warmouth sunfish, and yellow bullhead.

Large, algal-dominated, alkaline, highly conductive lakes with marine connections (Group 1) contained euryhaline species (Cluster I). The second fish group (Cluster II) consisted mainly of species that, while having a broad Atlantic Slope-Coastal Plain distribution, prefer acidic wetlands (Hastings 1979; Lee et al. 1980). The six lakes dominated by this assemblage were highly colored, weed dominated, and the second most acidic of all lake groups (Table 3).

Fish in Cluster III represented widely distributed species that occurred in lakes of many types, but comprised the major group in 12 lakes of the Florida panhandle. While their habitat requirements are less restricted than those of the previous two fish groups, Cluster III species prefer clearwater, less weeded lakes with a larger pelagic zone than Cluster III fish (Lee et al. 1980). The fourth fish assemblage dominated moderately productive, large, alkaline lakes lacking marine connection. Black crappie, two species of gar, bowfin, and two ictalurids were important components and typify the fish communities of lakes with well developed benthic food supplies (Lee et al. 1980; Learm and Freeman 1986). These same species also comprised a major portion of the fish fauna of large marine-influenced lakes (Apopka, Griffin, and Dora). Fish of all four clusters were found in these lakes reflecting the fact that large productive lakes provide more diverse habitats and greater food resources, and generally have a more diverse fish fauna than small lakes (Barbour and Brown 1974; Rahel 1984; Browne 1981).

The fish fauna of insular, small, acidic oligotrophic lakes are almost exclusively those taxa that can find an immigration route and withstand conditions that exclude many other species (Rahel 1984). Thus, the fact that the smallest, most acidic, least productive and most isolated lakes in our sample (Group 5) form a group containing the fewest fish species (mean = 7.44) and are not dominated by any one fish cluster is a reflection of the harsher conditions in those lakes. Among their fauna are some of the most acid-tolerant species (blue-spotted sunfish, swamp darter, largemouth bass, and bluegill).

Species Richness

Among the fourteen physico-chemical parameters examined, lake surface area was the best predictor of fish species richness in Florida. Lake size alone accounted for 45% of the variance in the species richness regression model (Table 5); this result was not unexpected since lake size has been identified as an important factor elsewhere (Barbour and Brown 1974; Eadie

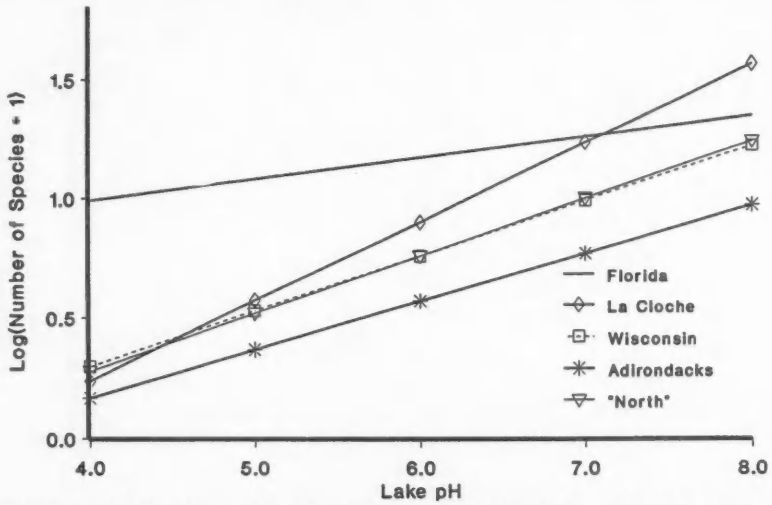

FIG. 1. Regression lines for number of fish species vs. pH for Florida, Wisconsin (Rahel 1982), La Cloche (Harvey 1975), Adirondack Mountain (Adirondack Lake Survey Corp. 1989) lakes, and the merged north-temperate data "North." All are significant ($P \leq 0.05$). F: $Y = 0.09$ (pH) + 0.63; $R^2 = 0.33$. C: $Y = 0.33$ (pH) − 1.08; $R^2 = 0.35$. W: $Y = 0.23$ (pH) − 0.62; $R^2 = 0.50$. NY: $Y = 0.20$ (pH) − 0.63; $R^2 = 0.43$. N: $Y = 0.24$ (pH) − 0.68; $R^2 = 0.38$.

et al. 1986; Rahel 1986; Beamish et al. 1976; Somers and Harvey 1984). Larger lakes generally have greater habitat heterogeneity (Emery 1978; Tonn and Magnuson 1982; Eadie et al. 1986; Barbour and Brown 1974) and a more diverse trophic structure than do small lakes (MacArthur 1965).

In Florida, larger lakes are generally more productive (lake size positively correlated with chlorophyll *a* ($r = 0.52$)). The greater abundance and diversity of food and habitat in large lakes can support more fish species (MacArthur 1965; Crisman et al. 1984) because of reduced competitive interaction among species. The species pool of large lakes having a marine connection can be further enhanced by euryhaline taxa.

Although lake pH was a significant predictor of fish species richness for Florida lakes, it was secondary to lake size. The change in species number with pH was very gradual in our lake set. Division of the lakes into three pH groups: 5.0–5.5, 4.5–4.9, and 4.0–4.4 (only one lake), yielded mean species numbers of 11.97 ± 1.41, 9.55 ± 1.36, and 28, respectively. These values are substantially higher than in acid-stressed La Cloche Mountain lakes (Harvey 1975) and Adirondack lakes (Adirondack Lake Survey Corp. 1989), and 138 Wisconsin lakes (Rahel 1984) spanning the same pH ranges. Unlike Florida, many lakes with pH less than 5.5 were fishless in the latter three regions.

The most striking results of the current study came from interregional comparisons of fish species richness with lake size and pH. Species richness at a given lake size was similar among the regions although small lakes in Florida had more species than did north-temperate lakes. However, differences in the relative response in species number for a given decrease along the pH axis (Fig. 1) between temperate and subtropical lakes was dramatic.

Possible factors contributing to the greater number of fish species at a given pH in Florida lakes, especiallly below 5.5 include: the presence of more acid-tolerant fish species in Florida, the effects of genetic adaptation of populations in low pH waters, lower dissolved aluminum concentrations, longer growing season, and lack of significant episodic acid inputs.

The fish fauna of peninsular Florida is dominated by centrarchids (17 species), ictalurids (eight species) and cyprinodontids (11 species). There is a paucity of species in fish families that dominate eastern North America: Cyprinidae, Percidae, and Catostomidae (Gilbert 1987). Many centrarchids (sunfish) and ictalurids (catfish) species have pH tolerances as low as 4.6 and often comprise the major species in temperate acid waters (Adirondack Lake Survey Corp. 1989; Rahel and Magnuson 1983; Learm and Freeman 1986; Frey 1951; Smith 1957; Harvey 1980). The most common cyprinid (minnow) in our lakes was the golden shiner which is also found in acidic temperate waters.

In addition to being naturally tolerant of low pH, *Lepomis macrochirus*, *Lepomis marginatus* and *Micropterus salmoides*, and *Ictalurus natalis* have undergone enough differentiation to be considered distinct from relatives elsewhere in eastern North America (Gilbert 1987). The first three are subspecies, while the latter is a separate race. Thus, there has been time enough to permit adaptation to acidic conditions. On a shorter term, however, there is evidence from studies in Florida (Canfield et al. 1985) and north-temperate regions (Rahel 1983; McWilliams 1980, 1982; Scherer et al. 1986) that fish in acidic waters can adapt physiologically, as evidenced by decreased blood ion losses relative to fish from alkaline waters, following experimental exposure to low pH. In some cases, decreased growth rates have been recorded for fish in acid lakes (Romie 1989; Wiener et al. 1984; Ryan and Harvey 1980; Keller 1984) but whether that is the result of acid-stress, food availability, or density dependent competition for food is not clear.

While increased levels of dissolved Na^+, K^+, Cl^-, or Ca^{2+} have decreased the loss of blood ions in fish experimentally exposed to acidic waters (Playle et al. 1989; McDonald 1983; McWilliams 1982), the concentrations of these ions in acidic Florida lakes (Garren et al. 1984; Canfield 1981; Hendry and

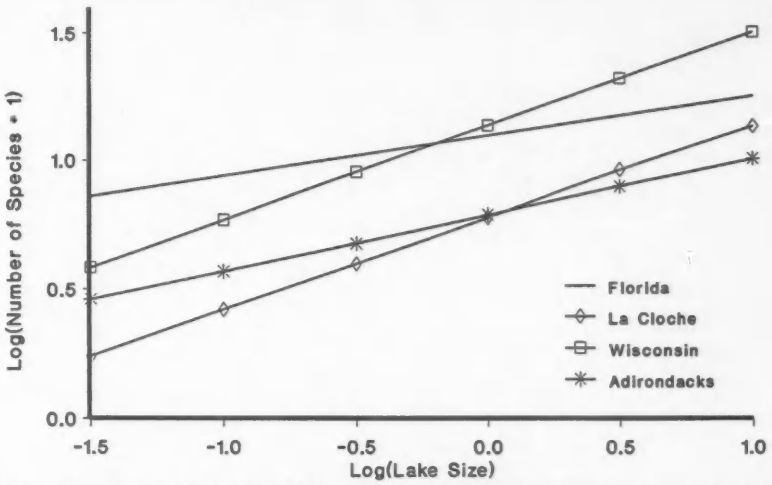

FIG. 2. Regression lines for number of fish species vs. lake surface area for Florida, Wisconsin (Rahel 1982), La Cloche (Harvey 1975) and Adirondack Mountain (Adirondack Lake Survey Corp. 1989) lakes. $Y = \text{Log}_{10}$ (Number fish species + 1). All are significant ($P \leq 0.05$). F: $Y = 0.16\ \text{Log}_{10}\ (\text{km}^2) + 1.10$; $R^2 = 0.43$. W: $Y = 0.37\ \text{Log}_{10}\ (\text{km}^2) + 1.14$; $R^2 = 0.69$. C: $Y = 0.36\ \text{Log}_{10}\ (\text{km}^2) + 0.78$; $R^2 = 0.29$. NY: $Y = 0.22\ \text{Log}_{10}\ (\text{km}^2) + 0.79$; $R^2 = 0.09$.

Brezonik 1984) are similar to those in acid-sensitive or acidic temperate lakes, e.g. Na^+ = 1–5 mg/L, Ca^{2+} = 1–4 mg/L (Beamish et al. 1976; National Research Council Canada 1981; Kanciruk et al. 1986). Therefore, dissolved ion levels cannot be responsible for the greater number of species in Florida lakes than in comparable temperate lakes.

Because of soil conditions (Byers 1984) even acidic Florida lakes have aluminum concentrations lower than the 200 μg/L level accepted as toxic to fish (Garren et al. 1984; Romie 1989; Hendry and Brezonik 1984). Values generally range from 20–180 μg/L for Florida lakes with pH as low as 3.7 (Lamia 1987; Romie 1989; Garren et al. 1984; Hendry and Brezonik 1984). Aluminum does not appear to be a factor in Florida lakes. Yet, we see a decrease in fish species richness with lower pH just as in acidified temperate lakes.

The impact of latitude on fish species richness has been recognized (Barbour and Brown 1974; Emery 1978; Eadie and Keast 1984). Since examinations of species relations to pH have most often been performed intra-regionally, it is not clear just how great a role or by what process latitudinal differences contribute to lowered species number at higher latitudes. Increased habitat heterogeneity and length of growing season (Emery 1978; Eadie and Keast 1984; Barbour and Brown 1974) have been suggested as contributors to higher species richness at lower latitudes. These factors may be important in explaining the presence of more fish species in Florida lakes at low pH than are found in even naturally acidic temperate lakes. Macrophytes are more consistently present in Florida lakes results in greater habitat diversity that can support more species (Crisman et al. 1984). This becomes even more important when comparing acidic lakes of these two regions because decreases in both primary (Almer et al. 1974; Kwiatkowski and Roff 1976) and secondary (Okland and Okland 1980; Wiederholm and Eriksson 1977) production may occur with increasing acidification. The former may be due to the decreased availability of nutrients at low pH (Fee 1980; Wilcox and DeCosta 1982). Several studies of Florida lakes have concluded that growth rates and conditions of fish were more related to productivity (chlorophyll *a*) than to pH (Lamia 1987; Romie 1989; Keller 1984; Canfield et al. 1985).

In north-temperate regions, concentrated acid and metal input from autumn rains and spring snowmelt have been suggested as factors leading to recruitment failure in fish species that spawn in fall and hatch in spring (Peterson et al. 1982). The fact that Florida fish spawn in spring and summer protects them from episodes of acid rain which may occur in Florida during the winter (Henry 1977). While this may contribute to a higher species number over time, acid precipitation has not made a significant impact in Florida yet (Sweets et al. 1989; Charles et al. 1986).

Interregional comparisons between lake size and fish species richness were inconclusive. While larger north-temperate and Florida lakes had more species than comparably sized La Cloche or Adirondack lakes, and more than small Wisconsin lakes. However, these differences were not statistically significant. The predominance of faunally depauperate bog lakes among the small lakes in the Wisconsin data may have skewed the regression and enhanced the apparent decrease in species number with lower lake size. Regardless, the underlying importance of lake size in determining species richness is evident and based on the similarity among the regions, size may be a more fundamental factor in determining fish species richness than pH. The two unacidified regions (Wisconsin and Florida) have higher intercepts than do the acidified regions representing the presence of a greater number of species in small to medium-sized lakes.

Thus, while fish species richness declines with pH in Florida lakes the decline is not solely the result of lake chemistry or the loss of particular species. Such observations suggest that factors structuring temperate fish communities in acidic lakes may be somewhat different from those underlying the response of fish in acidic Florida lakes.

Acknowledgements

We thank biologists Norman Young, Sam McKinney, Bill Johnson, and Jerry Krummrich of the Florida Game and Fresh Water Fish Com-

mission for giving us access to reports and unpublished data, and to the Adirondack Lake Survey Corporation for data they supplied. This research was partially funded by the Base Research Foundation.

References

ADIRONDACK LAKE SURVEY CORP. 1989. Adirondack Lakes Study 1984–1987. An evaluation of fish communities and water chemistry. Ray Brook, New York, NY.

ALMER, B., W. DICKSON, C. EKSTROM, E. HORNSTROM, AND U. MILLER. 1974. Effects of acidification on Swedish lakes. Ambio 3: 30–36.

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1981. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D. C.

BAKER, J. P. 1982. Effects on fish of metals associated with acidification, p. 165–176. *In* R. E. Johnson [ed.] Acid Rain/Fisheries, Proc. Int'l. Symp. Acid. Precip. Fish. Impacts Northeastern N.A., Northeastern Div. Am. Fish. Soc. Ithaca, NY.

BARBOUR, C . D., AND J. H. BROWN. 1974. Fish species diversity in lakes. Am. Nat. 108: 473–489.

BEAMISH, R. J., L. M. BLOUW, AND G. A. MCFARLANE. 1976. A fish and chemical study of 109 lakes in he Experimental Lakes Area (ELA), Northwestern Ontario, with appended reports on lake whitefish ageing errors and the northwestern Ontario baitfish industry. Fisheries and Marine Service, Tech. Rep. 607.

BROWNE, R. A. 1981. Lakes as islands: biogeographic distribution, turnover rates, and species composition in the lakes of central New York. J. Biogeography 8: 75–83.

BYERS, G. E. 1984. The effects of acid rain on the movement of ions in a typic quartzipsamment soil under natural vegetation in Florida. Ph.D. dissertation. University of Florida, Gainesville, FL. 310 p.

CANFIELD, D. E. 1981. Chemical and trophic state characteristics of Florida lakes in relation to regional geology. University of Florida, Florida Cooperative Fish and Wildlife Unit, Final Report, Gainesville, FL.

CANFIELD, D. E., JR., M. J. MACEINA, L. M. HODGSON, AND K. A. LANGELAND. 1983. Limnological features of some northwestern Florida lakes. J. Freshwater Ecol. 2: 67–79.

CANFIELD, D. E., JR., M. J. MACEINA, AND F. G. NORDLIE. 1985. Plasma osmotic and electrolyte concentrations of largemouth bass from some acidic Florida lakes. Trans. Am. Fish. Soc. 114: 423–429.

CHARLES, D. F., D. R. WHITEHEAD, D. S. ANDERSON, R. BIENERT, K. E. CAMBURN, R. B. COOK, T. L. CRISMAN, R. B. DAVIS, J. FORD, B. R. FRY, R. A. HITES, J. S. KAHL, J. C. KINGSTON, R. G. KREIS, JR., M. J. MITCHELL, S. A. NORTON, L. A. ROLL, J. P. SMOL, R. SWEETS, A. J. UUTALA, J. R. WHITE, M. C. WHITING, AND R. J. WISE. 1986. The PIRLA project (Paleoecological Investigation of Recent Lake Acidification): preliminary results for the Adirondacks, New England, N. Great Lakes States, and N. Florida. Water Air Soil Pollut. 30: 355–365.

CRISMAN, T. L., C. L. CLARKSON, A. E. KELLER, R. A. GARREN, AND R. W. BIENERT. 1984. A preliminary assessment of the importance of the littoral and benthic autotrophic communities in acidic lakes, p. 17–27. *In* B. G. Isom, S. D. Dennis, and J. M. Bates [ed.] Impact of Acid Rain and Deposition on Aquatic Biological Systems, ASTM STP 928. American Society for Testing and Materials, Philadelphia, PA.

DUNSON, W. A., F. SWARTS, AND M. SILVESTRI. 1977. Exceptional tolerance to low pH of some tropical blackwater fish. J. Exp. Zool. 201: 157–162.

EADIE, J. M., AND A. KEAST. 1984. Resource heterogeneity and fish species diversity in lakes. Can. J. Zool. 62: 1689–1695.

EADIE, J. M., T. A. HURLY, R. D. MONTGOMERIE, AND K. L. TEATHER. 1986. Lakes and rivers as islands: species-area relationships in the fish faunas of Ontario. Env. Biol. Fish. 15: 81–89.

EMERY, A. R. 1978. The basics of fish community structure: marine and freshwater comparisons. Env. Biol. Fish. 3: 33–47.

FEE, E. J. 1980. The importance of factors controlling annual phytoplankton production in the Experimental Lakes Area. Can. J. Fish. Aquat. Sci. 37: 513–522.

FLORIDA ACID DEPOSITION MONITORING PROGRAM. 1989. 1988 summary report. Hunter/ESE 91126-0106-3170.

FLORIDA GAME AND FRESH WATER FISH COMMISSION. 1960. Florida's Fresh Water Fishing: Choctawhatchee Watershed.

1978. Fish Management Report, Northwest Region.

FREY, D. C. 1951. The fishes of North Carolina's bay lakes and their intraspecific variation. Elisha Mitchell Sci. Soc. 67: 1–44.

FROMM, P. O. 1981. A review of some physiological and toxicological responses of freshwater fish to acid stress. Env. Biol. Fish 5: 79–93.

GARREN, R. A., C. L. CLARKSON, T. L. CRISMAN, AND S. M. ENGLISH. 1984. Limnological and bathymetric survey of 60 lakes in the Ocala National Forest, Florida. Final report submitted to U.S. Forest Service, Tallahassee, FL.

GILBERT, C. R. 1987. Zoogeography of the freshwater fish fauna of southern Georgia and peninsular Florida. Brimleyana 13: 25–54.

HARVEY, H. H. 1975. Fish populations in a large group of acid-stressed lakes. Verh. Int. Ver. Limnol. 19: 2406–2417.

1980. Widespread and diverse changes in the biota of North American lakes and rivers coincident with acidification, p. 93–98. *In* D. Drablos and A. Tollan [ed.] Ecological Impact of Acid Precipitation. Proceedings of an international conference, Sandefjord, Norway.

HARVEY, H. H., AND C. LEE. 1982. Historical fisheries changes related to surface water pH changes in Canada, *In* R. E. Johnson [ed.] Acid Rain/ Fisheries. American Fisheries Society, Bethesda, MD.

HASTINGS, R. W. 1979. Fish of the Pine Barrens, p. 489–504. *In* R. T. T. Forman [ed.] Pine Barrens: Ecosystem and Landscape. Academic Press, New York, NY.

HENDRY, C. D. 1977. The chemistry of precipitation in north-central Florida. M.S. thesis, University of Florida, Department of Environmental Engineering. 140 p.

HENDRY, C. D., AND P. L. BREZONIK. 1984. Chemical composition of softwater Florida lake and their sensitivity to acid precipitation. Water Resour. Bull. 20: 75–86.

HUBER, W. C., P. L. BREZONIK, J. P. HEANEY, R. E. DICKINSON, S. D. PRESTON, D. S. DWORNIK, AND M. A. DEMAIO. 1982. A classification of Florida lakes. Publ. 72, Water Resources Research Center, Department of Environmental Engineering Sciences, University of Florida, Gainesville, FL.

JENSEN, K. W., AND E. SNEKVIK. 1972. Low pH levels wipe out salmon and trout populations in southernmost Norway. Ambio 1: 223–225.

JEWELL, M. E., AND H. W. BROWN. 1929. Studies on northern Michigan bog lakes. Ecology 10: 427–475.

KANCIRUK, P., J. M. EILERS, R. A. MCCORD, D. H. LANDERS, D. F. BRAKKE, AND R. A. LINTHURST. 1986. Characteristics of Lakes in the Eastern United States Vol. III: data compendium of site characteristics and chemical variables. EPA/600/4-86/007c. U.S. Environmental Protection Agency, Washington, D.C.

KELLER, A. E. 1984. Fish communities in Florida lakes: relationship to physico-chemical parameters. M.S. thesis, University of Florida, Gainesville, FL. 106 p.

KELSO, J. R. M., AND C. K. MINNS. 1982. Current status of lake acidification and its effect on the fishery resources of Canada, p. 64–82. *In* R. E. Johnson [ed.] Acid Rain/Fisheries Am. Fish. Soc. Bethesda, MD.

KENNER, W. E. 1964. Maps Showing Depths of Selected Lakes in Florida. State of Florida, State Board of Conservation, Division of Geology. Florida Geological Survey, Inf. Circ. 40. U.S.G.S.

KWIATKOWSKI, R. E., AND J. C. ROFF. 1976. Effects of acidity on the phytoplankton and primary productivity of selected northern Ontario lakes. Can. J. Bot. 54: 2546–2561.

LAMIA, J. A. 1987. The limnological and biological characteristics of Cue Lake, an acidic lake in north Florida. M.S. thesis, University of Florida, Gainesville, FL. 116 p.

LEARM, J., AND B. J. FREEMAN. 1986. Fishes of the Okefenokee Swamp. University of Georgia Press, Athens, GA.

LEE, D. S., C. R. GILBERT, C. H. HOCUTT, R. E. JENKINS, D. E. MCALLISTER, AND J. R. STAUFFER, JR. 1980. Atlas of North American Freshwater Fishes. N.C. Mus. Nat. Hist., Raleigh, NC. 854 p.

LINTHURST, R. A., D. H. LANDERS, J. M. EILERS, D. F. BRAKKE, W. S. OVERTON, E. P. MEIER, AND R. E. CROWE. 1986. Characteristics of Lakes in the Eastern United States. Vol. I: Population descriptions and physico-chemical relationships. EPA/600/4-86/700a, U.S. Environmental Protection Agency, Washington, D.C.

MACARTHUR, R. H. 1965. Patterns of species diversity. Biol. Rev. 40: 510–533.

MCDONALD, D. G. 1983. The effects of H^+ upon the gills of freshwater fish. A review. Can. J. Zool. 61: 691–703.

MCWILLIAMS, P. G. 1980. Acclimation to an acid medium in the brown trout, *Salmo trutta*. J. Exp. Biol. 88: 269–280.

1982. A comparison of physiological characteristics in normal and acid exposed populations of the brown trout *Salmo trutta*. Comp. Biochem. Physiol. 72A: 515–522.

MYERS, G. S. 1938. Fresh-water fishes and West Indian zoogeography. Ann. Rep. Smith. Inst. 1937. Washington, D.C. p. 339–364.

NATIONAL RESEARCH COUNCIL CANADA. 1981. Acidification in the Canadian aquatic environment: scientific criteria for assessing the effects of acidic

deposition on aquatic ecosystems. Associate Committee On Scientific Criteria For Environmental Quality, Ottawa. p. 179–182.

NIE, H. 1975. SPSS: Statistical Package for the Social Sciences. McGraw-Hill Inc., New York, NY.

NORTON, S. A., AND R. B. DAVIS. 1981. Responses of Maine lakes to atmospheric inputs of acids and heavy metals: final Rep., U.S. Office of Water Research and Technology, Project A-048-ME.

OKLAND, J., AND K. A. OKLAND. 1980. pH level and food organisms for fish — studies of 1,000 lakes in Norway, p. 326–328. *In* D. Drablos and A. Tollan [ed.]. Proc. Int. Conf. Ecological Impact Acid Precipitation, Norway, SNSF Project, March 11–14, 1980.

PETERSON, R. H., P. G. DAYE, G. L. LACROIX, AND E. T. GARSIDE. 1982. Reproduction in fish experiencing acid and metal stress, p. 177–196. *In* R. E. Johnson [ed.] Acid Rain/Fisheries. American Fisheries Society, Bethesda, MD.

PIEFFER, M., AND P. FESTA. 1980. Acidity status of lakes in the Adirondack region of New York in relations to fish resources. N.Y. Dep. Env. Conserv. Rep. FW-P168 (10/80).

PLAYLE, R. C., G. G. GOSS, AND C. M. WOOD. 1989. Physiological disturbances in rainbow trout (*Salmo gairdneri*) during acid and aluminum exposures in soft water of two calcium concentrations. Can. J. Zool. 67: 314–324.

RAHEL, F. J. 1982. Fish assemblages in Wisconsin bog lakes. Ph.D. dissertation. University of Wisconsin, Madison, WI.

1983. Population differences in acid tolerance between yellow perch, *Perca flavescens*, from naturally acidic and alkaline lakes. Can. J. Zool. 61: 147–152.

1984. Factors structuring fish assemblages along a bog lake successional gradient. Ecology 65: 1276–1289.

1986. Biogeographic influences on fish species composition of northern Wisconsin lakes with applications for lake acidification studies. Can. J. Fish. Aquat. Sci. 43: 124–134.

RAHEL, F. J., AND J. J. MAGNUSON. 1983. Low pH and the absence of fish species in naturally acidic Wisconsin lakes: inferences for cultural acidification. Can. J. Fish. Aquat. Sci. 40: 3–9.

ROMIE, K. F. 1989. Trophic status and fish populations in five lakes of low pH in northern Florida. M.S. thesis, University of Florida, Gainesville, FL. 115 p.

RYAN, P. M., AND H. H. HARVEY. 1977. Growth of the rock bass, *Ambloplites rupestris*, in relation to the morphoedaphic index as an indicator of environmental stress. Can. J. Fish. Aquat. Sci. 34: 2079–2088.

1980. Growth responses of yellow perch, *Perca flavescens*, to lake acidification in the La Cloche Mountain Lakes of Ontario. Env. Biol. Fish. 5: 97–108.

SAS. 1982. SAS User's Guide. SAS Institute. Cary, N. C. Sarle, W. S. 1983. The cubic clustering criterion. Tech. Rep. A-108. SAS Institute, Inc. Carey, N.C.

SCHERER, E., S. E. HARRISON, AND S. B. BROWN. 1986. Locomotor activity and blood plasma parameters of acid-exposed lake whitefish, *Coregonus clupeaformis*. Can. J. Fish. Aquat. Sci. 43: 1556–1651.

SCHOFIELD, C. L. 1976. Acid precipitation: effects on fish. Ambio 5: 228–230.

1977. Acidification of Adirondack lakes by acid precipitation: extent and magnitude of the problem. F-28-R Fed. Aid Final Rep.

1982. Historical fisheries changes in the United States related to decreases in surface water pH, p. 57–68. *In* R. E. Johnson [ed.] Acid/Rain Fisheries. American Fisheries Society, Bethesda, MD.

SOMERS, K. M., AND H. H. HARVEY. 1984. Alteration of fish communities in lakes stressed by acid deposition and heavy metals near Wawa, Ontario. Can. J. Fish. Aquat. Sci. 41: 20–29.

SMITH, R. F. 1957. New Jersey Fisheries Survey. The State of New Jersey, Department of Conservation and Economic Development, Division of Fish and Game. Trenton, N.J.

SWEETS, R., R. W. BIENERT, T. L. CRISMAN, AND M. W. BINFORD. 1989. Paleoecological investigations of recent lake acidification in northern Florida. J. Paleolimno.

TONN, W. M., AND J. J. MAGNUSON. 1982. Patterns in the species composition and richness of fish assemblages in northern Wisconsin lakes. Ecology 63: 1149–1166.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA). 1979. Methods for chemical analysis of water and wastes. Environmental Monitoring and Support Laboratory, Office of Research and Development, United States Protection Agency, Cincinnati, OH.

WEINER, J. G., P. J. RAGO, AND J. M. EILERS. 1984. Species composition of fish communities in northern Wisconsin lakes: relation to pH, p. 133–146. *In* G. R. Hendrey [ed.] Early Biotic Responses To Advancing Lake Acidification. Butterworth Publishers, Boston, MA.

WOOD, C. M., AND D. G. MCDONALD. 1982. Physiological mechanisms of acid toxicity to fish, *In* Acid Rain/Fisheries. American Fisheries Society, Bethesda, MD.

WIEDERHOLM, T., AND L. ERIKSSON. 1977. Benthos of an acid lake. Oikos 29: 261–267.

WILCOX, G., AND J. DECOSTA. 1982. The effect of phosphorus and nitrogen addition on the algal biomass and species composition of an acidic lake. Arch. Hydrobio. 94: 393–424.

WRIGHT, R. F., AND E. SNEKVIK. 1978. Acid precipitation: chemistry and fish populations in 700 lakes in southernmost Norway. Verh. Int. Ver. Limnol. 20: 765–775.

WRIGHT, R. R., N. CONROY, W. T. DICKSON, R. HARRIMAN, A. HENRIKSEN, AND C. L. SCHOFIELD. 1981. Acidified lake districts of the world: a comparison of water chemistry of lakes in southern Norway, southern Sweden, south-western Scotland, the Adirondack Mountains of New York, and southeastern Ontario, p. 377–379. *In* D. Drablos and A. Tollan [ed.] Proc. Int. Conf. Ecological Impact Acid Precipitation, Norway, SNSF Project, March 11–14, 1980.

Appendix A

Chemical and physical characteristics of 36 Florida lakes used in this study. Reference: 1 = Garren et al. 1984; 2 = Hendry 1980; 3 = Florida Game and Fresh Water Fish Commission (FGFWFC); 4 = Canfield 1981. All fish species numbers are based on sampling by the FGFWFC. SD = Secchi depth (m); Con = Specific conductivity (μmhos/cm); SA = surface area (km^2); Chla = Chlorophyll *a* (μg/L); Color = mg/L as Pt-Co; Species = number of fish species collected. Alk = alkalinity (mg/L $CaCO_3$); TN = Total Kjeldhal nitrogen (mg/L); TP = Total phosphorus (mg/L); Ca = calcium (mg/L); Na = sodium (mg/L); K = Potassium (mg/L). Missing data designated by a "—".

Number Lake	pH	Ca	Na	K	TN	TP	Alk	Chla	SD	SA	Color	Cond	Species	Al	% Cover	Reference
1. Altho	5.99	2.48	5.07	0.64	0.57	0.029	2.7	5.51	1.00	2.7	194	582	28	0	9	2
2. Doe	4.59	0.89	4.13	0.18	0.18	0.008	1.8	0.86	4.10	0.4	5	47	6	42.5	—	1
3. Apopka	8.87	38.48	19.30	10.00	4.59	0.166	131.0	156.10	0.20	129.5	37	340	23	—	4	4
4. Bear	4.50	0.28	2.30	0.20	0.34	0.015	2.0	10.90	1.70	0.4	30	16	11	—	40	4
5. Buckskin		0.91	2.76	0.22	0.73	0.011	1.5	1.08	2.10	0.1	21	30	7	—	100	1
6. Catherine	4.66	0.67	4.07	0.30	0.44	0.007	1.8	0.71	4.30	0.5	13	39	8	20	80	1
7. ChainOLks	4.84	0.71	3.49	0.33	1.23	0.16	2.0	1.36	2.60	0.7	12	41	14	20	<5	1
8. Compass	5.22	1.16	1.60	0.30	0.16	0.005	2.0	0.70	4.90	2.4	1	18	9	—	20	4
9. Cowpen	4.74	0.78	5.49	0.21	0.66	0.10	1.5	1.03	1.50	0.2	33	47	5	20	80	1
10. Dora	8.91	37.04	16.80	9.60	3.06	0.09	120.0	123.50	0.40	17.9	43	321	31	—	2	4
11. Dunford	4.96	0.64	1.70	0.20	0.22	0.007	1.0	0.80	5.00	1.8	6	15	8	—	—	4
12. Eaton	6.51	10.18	4.14	0.24	1.16	0.046	11.5	0.93	0.30	1.2	613	89	17	—	36	1
13. Gap Pond	5.12	0.64	1.70	0.20	0.31		1.0	1.50	4.50	3.4	4	13	18	—	40	3
14. Griffin	8.63	29.79	14.60	4.80	2.17	0.083	96.0	72.80	0.40	35.6	23	261	33	—	<1	4

Appendix A *(concluded)*

Number Lake	pH	Ca	Na	K	TN	TP	Alk	Chla	SD	SA	Color	Cond	Species	Al	%	Cover Reference
15. Hurricane	5.68	0.44	2.00	0.20	0.31	0.011	3.0	2.70	3.50	1.4	16	14	14	—	25	4
16. Iamonia	5.63	0.88	1.60	0.20	0.43	0.007	3.0	7.80	2.20	23.3	29	18	15	—	28	4
17. Juniper	5.47	0.48	1.80	0.20	0.39	0.020	2.0	7.50	2.40	2.7	17	13	12	—	30	4
18. Karick	6.01	1.84	2.00	0.30	0.24	0.019	9.0	3.80	2.90	0.2	20	28	13	—	10	4
19. Koon	5.11	1.48	2.60	0.10	0.63	0.009	3.0	5.60	1.30	0.4	117	23	17	—	100	4
20. Lochloosa	7.37	9.28	5.60	0.30	1.19	0.036	23.0	32.00	0.70	23.1	61	77	19	—	49	4
21. Lowry	5.06	5.28	13.60	0.10	1.10	0.022	5.0	2.00	1.40	5.1	79	23	7	38.0	<5	4
22. Magnolia	5.10	0.68	2.50	0.10	0.10	0.013	1.0	1.40	4.80	0.8	3	23	10	37.2	1	4
23. McCloud	4.71	0.95	2.79	0.59	0.38	0.016	0.5	0.88	3.80	0.1	15	45	6	72.5	2	2
24. Munson	7.72	17.64	16.10	2.80	2.93		58.0	144.90	0.40	1.0	51	187	11	—	16	4
25. Ocean	4.01	1.08	4.10	0.10	0.22	0.023	0.0	3.30	1.10	7.0	50	35	27	—	<1	4
26. Owens	5.00	3.50	4.90	0.70	0.58	0.030	2.0	—	—	0.3	—	—	15	—	—	3
27. Pate Pond	4.62	0.56	1.90	0.20	0.29	0.014	0.0	5.70	1.70	1.6	36	20	7	—	10	2
28. PorterPond	4.56	0.10	1.05	0.20	0.22	0.008	0.0	0.55	2.20	4.9	—	—	12	—	65	3
29. Round	6.30	1.88	1.90	0.40	0.17	0.005	0.0	0.80	4.70	0.1	0	2	12	—	20	4
30. Sampson	6.88	12.00	12.00	0.70	0.52	0.034	10.0	3.40	2.00	8.8	67	124	13	—	5	4
31. Sante Fe	6.02	2.12	6.80	0.30	0.36	0.016	3.0	5.40	2.20	19.1	33	24	18	—	1	4
32. So.Grass.	4.72	0.90	4.04	0.20	0.35	0.015	1.3	0.86	3.30	0.6	9	36	12	20	100	1
33. Stone	5.20	2.58	2.01	0.40	0.45	0.034	2.0	3.20	—	0.5	—	—	11	—	25	3
34. Victor	5.69	2.32	1.80	0.50	0.29	0.012	6.0	2.80	2.70	0.5	15	25	6	—	20	4
																1
35. Wildcat	4.54	0.60	3.95	0.18	0.26	0.006	1.8	1.13	3.50	1.1	3	43	14	20	18	
36. Buck	5.19	1.22	3.13	0.19	0.51	0.011	2.8	1.75	3.10	0.2	27	34	6	20	50	1

Intrabiome Distributional Patterns of Lotic Macroinvertebrate Assemblages

Lynda D. Corkum

Department of Biological Sciences, University of Windsor, Windsor, Ontario N9B 3P4 Canada

Corkum, L. D. 1990. Intrabiome distributional patterns of lotic macroinvertebrate assemblages. Can. J. Fish. Aquat. Sci. 47: 2147–2157.

Macroinvertebrates were collected at five sites along each of three rivers within the eastern deciduous forest biome in spring, summer, and autumn. Macroinvertebrate density at sites in mixed land use areas was intermediate between high levels at sites in farmlands and low levels in forests. Cluster analysis using mean density of the 24 dominant taxa revealed three groupings of river sites each of which was consistently associated with a land use type. Clusters were poorly associated with season of collection, site location along rivers, and with specific river drainage. Hydropsychidae and Leptophlebiidae characterized forested sites. The assemblage associated with mixed land use areas included Psephenidae, Baetidae, Tricorythidae, and Heptageniidae. Farmland river sites were characterized by Ceratopogonidae, Corixidae, Oligochaeta, and Tricladida. The mixed land use group of river sites was faunistically more similar to sites in forested than in farmland areas. Forested sites were fast-flowing and had little benthic detritus; farmland sites were slow-flowing and had high levels of detritus. The biome dependency hypothesis, which predicts that similar assemblages of macroinvertebrates are likely to occur at river sites both within and among drainages if the basins occupy the same biome, was not largely supported because characteristic vegetation had been disturbed.

Des macro-invertébrés ont été recueillis au printemps, à l'été et à l'automne à cinq sites de trois cours d'eau passant par le biome de la forêt orientale à feuilles caduques. La densité des macro-invertébrés dans les sites des zones où l'on fait un usage mixte des terres se situait à mi-chemin entre les fortes densités des sites des zones agricoles et les faibles densités des sites des zones forestières. L'analyse en grappes sur la base d'une densité moyenne de 24 taxons dominants a fait ressortir trois groupes de sites de rivières qui ont été chacun systématiquement associés à un type d'utilisation des terres. Les grappes n'ont été que faiblement associées avec la saison de collecte, l'emplacement des sites le long des cours d'eau et le débit de ceux-ci. On a surtout capturé des hydropsychidés et des leptophlebiidés dans les sites forestiers. Dans les zones faisant l'objet d'une utilisation mixte, on retrouvait surtout des psephenidés, des baetidés, des tricorythidés et des heptageniidés. Les sites des zones agricoles étaient dominés par des ceratopogonidés, des corixidés, des oligochaetidés et des tricladidés. La faune des groupes de sites choisis dans la partie d'utilisation mixte des sols rssemblait davantage à celle des sites des zones forestières qu'à celles des zones agricoles. Les sites des zones forestières se trouvaient à des endroits où le débit est rapide et les quantités de détritus benthiques y étaient faibles; les sites des zones agricoles étaient quant à eux à des endroits où le débit est lent et l'on y trouvait des quantités importantes de détritus. L'hypothèse de dépendance face au biome, qui précise que l'on trouvera très probablement des assemblages similaires de macro-invertébrés le long des rivières au sein de mêmes bassins versants et entre les bassins versants si ceux-ci occupent le même biome, n'a pas été véritablement confirmée parce que la végétation typique a été perturbée.

Received February 15, 1990
Accepted June 6, 1990
(JA472)

Reçu le 15 février 1990
Accepte le 6 juin 1990

One goal in ecology is the identification of factors that determine the abundance and distributional patterns of organisms (Andrewartha and Birch 1954, 1984). Historically, the described empirical relationships between riverine fauna and environmental factors were based on the classification of rivers partitioned into discrete units or zones from headwaters to mouth (Hawkes 1975). However, predictability of such models was poor when relationships were applied to rivers away from the original study area. The applicability of relationships to other study areas depends on environmental components and scale (Seifert 1984) as well as taxonomic resolution.

More recently, the importance of riparian vegetation and adjacent forests has been recognized in terms of energy inputs to streams (Fisher and Likens 1973; Hynes 1975). In developing the river continuum concept, Vannote et al. (1980) used drainage basin as a framework within which a continuously integrated series of physical gradients along a river were associated with changes in functional feeding groups of macroinvertebrates. They suggested that changes in feeding guilds of macroinvertebrates reflected the relative contribution of autochthonous and allochthonous inputs and transport of organic matter along rivers that originate in forested areas. Accordingly, several workers have emphasized the importance of riparian vegetation in structuring macroinvertebrate communities in streams (Cummins et al. 1984, 1989); however, the influence of the land (throughout the drainage basin) on stream biota has received less attention (but see Woodall and Wallace 1972; Molles 1982; Whittier et al. 1988). Additionally, the seasonal influence of climate and terrestrial vegetation on the life

history strategies of lotic macroinvertebrates at north temperate latitudes has been well documented (Hynes 1970; Rosenberg 1979). Changes in numbers and biomass of invertebrate taxa that follow particular life history strategies reflect latitudinal or altitudinal changes in temperature and available food sources (Clifford 1982).

Corkum (1989) evaluated the relative importance of biogeographical (landscape) and on-site hydrological variables on the spatial distribution of benthic macroinvertebrates at 100 river sites throughout northwestern North America. Although both types of variables contributed to the correct classification of site groupings characterized by distinct fauna, landscape features were more useful than hydrological variables in discriminating among site groupings. Such findings are of value in ecology because they permit one to anticipate the community composition of macroinvertebrates occurring at river sites from landscape information on maps.

Ross (1963) showed that there was a strong correspondence between distributional patterns of trichopteran larvae in small rivers and the terrestrial biome (defined by climate, but reflected by vegetation). Some caddisfly species were endemic to rivers in the western montane forests, whereas other species occurred only in rivers of the eastern deciduous forests. Although biomes appear to be an important determinant of broad-scale distributional patterns of lotic macroinvertebrates (Ross 1963; Wiggins and Mackay 1978; Corkum 1989), local and regional land use also influence the biotic structure and function of streams (Resh et al. 1988).

Expanding on earlier work by Ross (1963), Corkum (1989) suggested that similar assemblages of macroinvertebrates were most likely to occur at river sites within or among drainage basins if the basins occupied the same biome. This idea of similarity of macroinvertebrate distributional patterns within and among drainage basins differs from the more accepted view of faunal changes occurring along the length of a river (Hawkes 1975; Vannote et al. 1980).

In the present study, I examine whether or not significant, consistent differences occur among macroinvertebrate communities at river sites within a biome. A subsequent study will compare spatial patterns of macroinvertebrate communities in streams within and among biomes. I sampled macroinvertebrates and measured associated environmental factors at five sites along each of three rivers flowing through agricultural croplands and forested areas in the eastern deciduous forest biome during spring, summer, and autumn. If distributional patterns of lotic macroinvertebrate assemblages do differ among river sites within a biome, I wished to determine whether the patterns most strongly reflect (a) the season during which animals were collected; (b) the relative position of a site along the length of river; (c) the land through which rivers flow; or (d) the river within the biome. Using multivariate statistics, I identified groups of river site samples characterized by distinct macroinvertebrate assemblages, and examined each group for consistency with respect to sample season, site location, type of land use and river. I then evaluated which environmental variables best delineated the groups characterized by the assemblages.

Materials and Methods

Study Area

The Credit, Ausable, and Maitland rivers, located in southwestern Ontario, were each sampled at five locations along their length in spring (12–20 May), summer (20–24 July) and autumn (6–9, 16–17 October) 1987 (Fig. 1). The three drainages occur within the eastern deciduous forest biome (Udvardy 1975; Danks 1979).

These rivers were chosen because they were not interrupted by reservoirs and no major industrial development occurred within the drainage basins. Towns were present in all drainages, but urban centres were restricted to areas below the most downstream sampling location. All drainages were located on Palaeozoic (sedimentary) bedrock (Chapman and Putnam 1984). In addition, drainage basins were selected within a limited latitudinal range (43°04′ to 43°54′N) (Fig. 1) to reduce variation in life history development for any given taxon.

The Credit River, which drains an area of 843 km^2, rises in a hilly area of moraines and flows south through deciduous forests for about 95 km before entering Lake Ontario at Port Credit west of Toronto. The Credit River drops 415 m from its source to Lake Ontario at an average slope of 0.0044 m/m. The Ausable River, which drains an area of 1645 km^2, follows a depression in front of a glacial moraine (Chapman and Putnam 1984) and flows into Lake Huron at Grand Bend. The river drops 149 m from its source to Lake Huron at an average slope of 0.0020 m/m. The Maitland River drains an area of 2521 km^2 of glacial till and clay plain and enters Lake Huron at Goderich, north of Grand Bend. I sampled the main, northern branch of the Maitland River, which flows for about 144 km. Over this distance, the river drops 272 m from its source to the lake at an average slope of 0.0020 m/m. Both the Ausable and Maitland rivers flow through agricultural croplands.

Sample sites (numbered sequentially from upstream [site 1] to downstream [site 5]) were selected along river lengths (Fig. 2). Site locations corresponded whenever possible to gauging stations (Water Resouces Branch, Environment Canada). By chance, most river sites were sampled at low flow in spring, summer, and autumn (Fig. 3). However, during the summer, two downstream sites (4 and 5) on the Credit River were sampled 1 d after a local storm.

River widths at sample sites ranged from 6 to 75 m, corresponding to stream orders 3–7 (cf. Cummins 1975a). Recently, Hughes and Omernik (1981) stressed the imprecision of using stream order to represent size and width of rivers. Moreover, the effect of map scale on stream order (especially on low order streams) is well known (Leopold et al. 1964). Accordingly, I elected to use stream width rather than order as it is a direct and easily obtained measure of river size.

Sampling Procedures

River sites were sampled (within a 50 m section of river length) in spring, summer, and autumn. Because of growth and emergence of various aquatic insects (insects dominated the samples) throughout the year, I assumed that the 45 collections (15 river sites × 3 seasons) were independent.

The on-site environmental variables selected for measurement (minimum and maximum water temperature, conductivity, pH, dissolved oxygen, suspended sediments, ash free dry mass of sample detritus, river width, substrate type, riparian cover, bank stability, mean depth, mean current velocity) were shown previously to be important in the distribution of macroinvertebrates (Hawkes 1975; Pennak 1977; Vannote et al. 1980).

Maximum and minimum water temperatures were obtained for each site in each season sampled. At each sample site, a maximum-minimum thermometer was set in the river and

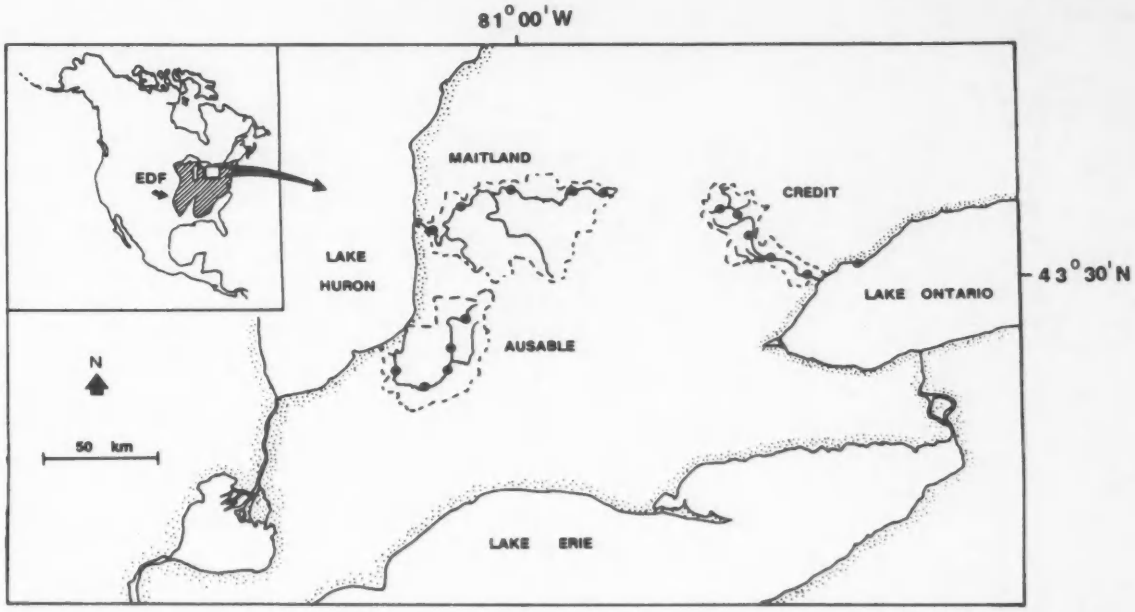

FIG. 1. Map of the Credit, Ausable, and Maitland drainage basins. Sample sites within the basins are indicated by closed circles. The drainage basins are within the eastern deciduous forest (EDF) biome of North America.

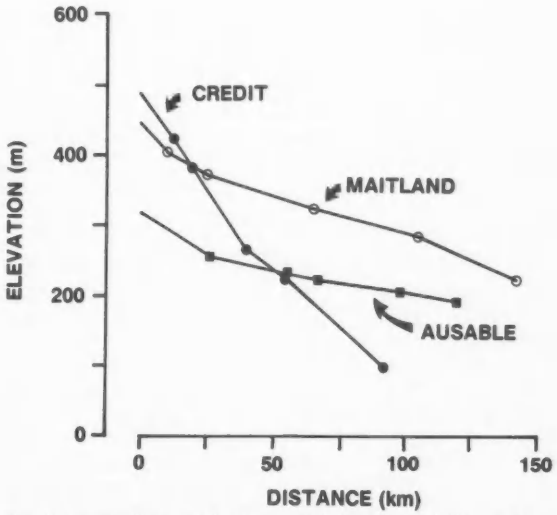

FIG. 2. Longitudinal profiles in terms of elevation and distance from source for the five study site locations on each river (Credit River, closed cicles; Maitland River, open circles; Ausable River, closed squares).

retrieved 1 d later to determine the diel range of water temperature.

In situ values for conductivity (Yellow Springs Instrument Co., YSI; model 33), pH (Can Lab digital pH meter, model 607) and dissolved oxygen concentrations (YSI oxygen meter, model 57) were obtained prior to sampling the benthos. Triplicate water samples were collected for analysis of suspended sediments. In the field, a known volume of water was filtered through preweighed, glass fiber GF/C filters and the filters were stored on dry ice. Subsequently, filters (including a control filter wetted with distilled water) were air dried (6 h), oven dried (103°C, 1 h), weighed, ashed (550°C, 20 min) and re-weighed to determine concentrations of suspended sediments (American Public Health Association 1985).

Macroinvertebrate samples were taken using a modified Hess cylinder sampler (0.1 m^2) with a 1-m trailing bag (mesh size: 250 μm). Five samples were collected along one transect and preserved with Kahle's fluid (Pennak 1978). In those cases where deep water prevented the collection of samples across an entire channel, samples were taken along two to three partial transects perpendicular to shore. To determine the ash free dry mass (AFDM) of detritus (the particulate organic matter remaining after the animals were retrieved from each sample in the laboratory), samples were air dried for 24 h, oven dried (103°C, 24 h), weighed, ashed (550 ± 25°C, 3 h) and re-weighed.

At each Hess sample point, depth and mean current velocity within the water column were determined (Price Gurley meter during the spring; Ott C2 meter in summer and autumn). Stream width and an overall qualitative assessment of predominant substrate type at river sites were noted. A description of the surrounding land use, riparian vegetation and bank stability, and percent cover or shading over the river also were recorded.

Land use was documented in upstream and downstream directions for as far as I could see. Photographs were taken in upstream and downstream directions at each site in all seasons. I noted any change in vegetation between the river valley and the plateau above. After the first sampling trip, it was clear that

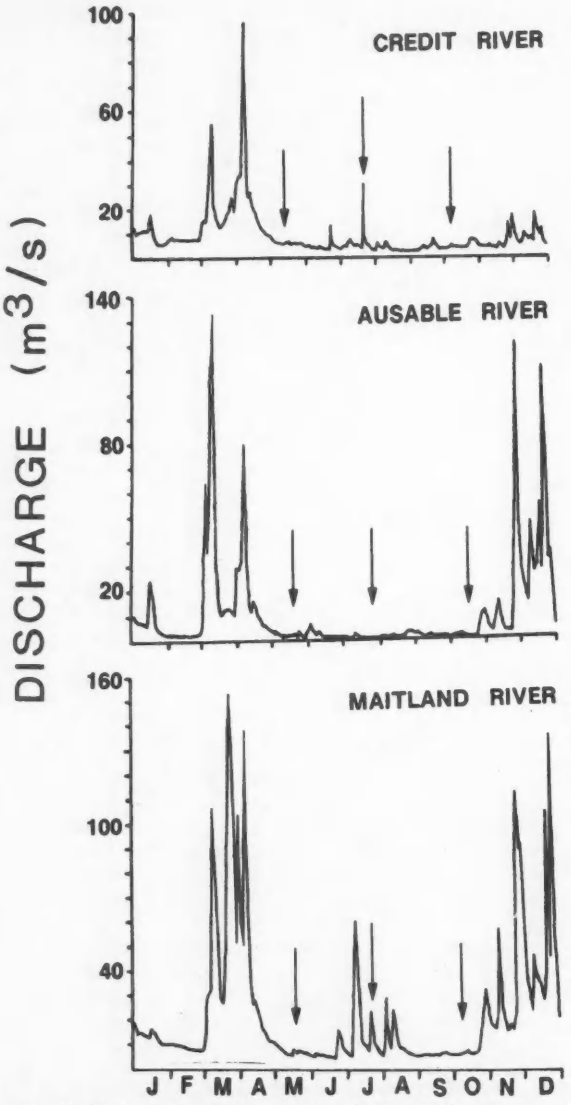

FIG. 3. Discharge data (1987) were provided by the Water Survey of Canada (WSC), Environment Canada, for the most downstream gauging station on each river: Credit River at Erindale (site 5), WSC station no. 02HB002; Ausable River near Springbank (site 4), WSC station no. 02FF002; Maitland River below Wingham (21 km upstream from site 4), WSC station no. 02FE002. The 1987 mean annual flow at each location was 8.98 m^3/s (Credit River), 10.4 m^3/s (Ausable River) and 20.0 m^3/s (Maitland River). Arrows represent sampling dates.

there were three types of land use associated with the study sites selected for study: forested, farmland, and mixed. Mixed land use areas were forested river valleys in farmlands. The 15 river sites were assigned (before macroinvertebrate samples were analysed) to land use types on the basis of these field observations: forested (Credit River sites 1, 2, 3, 4, 5), farmland (Ausable River sites 1, 2; Maitland River sites 1, 2, 3), and mixed (Ausable River sites 3, 4, 5; Maitland River sites 4, 5). One forest site (Credit River site 5) was best described as a forested river valley in an urban locale.

Taxonomic Resolution

Macroinvertebrates can be identified using various biological classifications (size, behaviour, functional feeding group, taxonomic composition) each of which can be related to a set of environmental factors (Corkum and Ciborowski 1988). In this study, I chose to work with taxonomic designations because such categorizations reflect broad environmental requirements as well as the biogeography of constituent groups. However, other biological classifications also could have been used to test the biome dependency hypothesis, recognizing that different categorizations depend on the scale of resolution. For example, some familial classifications correspond well with trophic assignments (e.g. Tricorythidae, Ephemeroptera; all species are collector-gatherers), yet there are problem groups (e.g. Chironomidae, Diptera) in which animals assigned to one familial classification may have representatives belonging to several different trophic levels.

Since distributional patterns of species are a function of biotic interactions, dispersal mechanisms, environmental tolerances and historical factors (Carter et al. 1980), the likelihood of a species being present throughout a large geographical area is low. Earlier, Illies (1961) associated families of aquatic insects with particular river zones (rhithron, potamon) on a world-wide basis. I also chose to compare macroinvertebrate communities among drainage basins using a familial classification.

In the laboratory, each benthic sample was washed through a standard (Tyler®) sieve series (mesh openings: 4.00–0.25 mm). Macroinvertebrates were sorted from the detritus in each size class and identified using a dissecting microscope. Insects were identified generally to familial level. Coarser designations were used for non-insect taxa (Tricladida, Pulmonata, Hirudinea, Branchiobdellida, Oligochaeta, Hydracarina, Amphipoda). Microcrustaceans were not retrieved from samples. Absolute density (number 0.1 m^{-2}) was determined for the entire sample.

Data Analyses

A total of 225 macroinvertebrate samples (3 seasons × 3 rivers/season × 5 sites/river × 5 samples/site) was included in the analysis. A replicated, three-way analysis of variance (ANOVA) (Sokal and Rohlf 1981) was used to test for significant differences in macroinvertebrate density (numbers/m^2) among rivers, site distances along rivers and seasons.

Ward's hierarchical method of cluster analysis (Wishart 1987) was used to group river collections in all three seasons (45 collections: 15 sites × 3 seasons/site) according to the mean ($n = 5$) density of each of 24 dominant taxa. A taxon was considered to be dominant if it accounted for at least 1% of the total density by number of all samples or 1% of any one of the 225 samples.

A series of ANOVA and multiple comparison tests was used to identify taxa that best characterized or defined the collection groupings derived from the cluster analysis. Twenty-four, one-way ANOVA tests (one per dominant taxon) were conducted to detect differences in mean density among the group clusters. If significant differences were observed among group means, a Student–Newman–Keuls (SNK) multiple comparison test was used to determine which group means (if any) did not differ from one another.

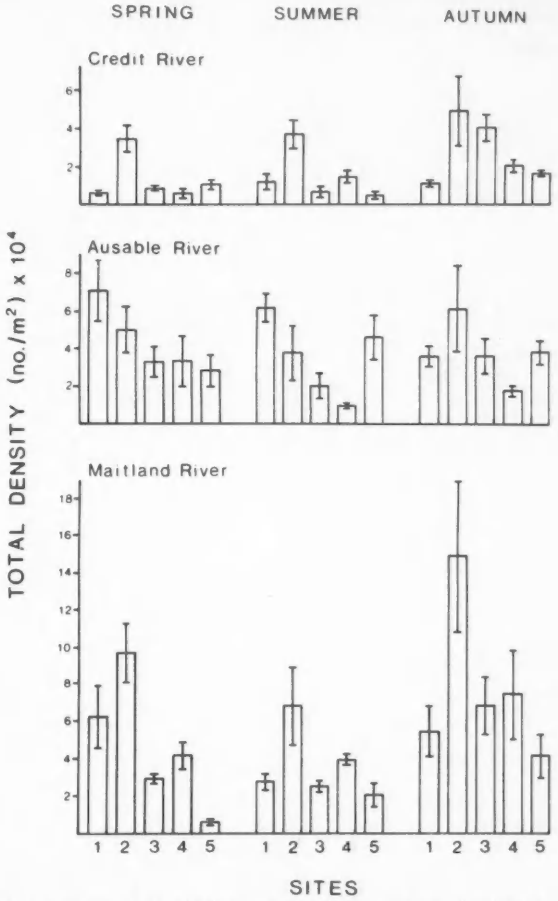

FIG. 4. Mean and standard errors of benthic invertebrate density (n = 5 samples) for each of five sites on the Credit, Ausable, and Maitland Rivers in spring, summer, and autumn. Sites are numbered from upstream (site 1) to downstream (site 5) locations.

TABLE 1. Summary of a three-way analysis of variance (ANOVA) test to examine the influence of rivers, site distance along rivers and seasons as well as the interactions of these factors on differences among mean benthic density.

Source of variation	df	SS	MS	F	P
Rivers	2	14.7883	7.3942	17.934	<0.001
Site distance	4	31.2052	7.8013	18.921	<0.001
Seasons	2	54.4733	27.2366	66.060	<0.001
Rivers × Site distance	8	8.8975	1.1122	2.698	<0.01
Rivers × Seasons	4	6.1716	1.5429	3.742	<0.01
Site distance × Seasons	8	23.8593	2.9824	7.234	<0.001
Rivers × Site distance × Seasons	16	18.5328	1.1583	2.809	<0.001
Within group (error)	180	74.2073	0.4123		
Total	224	232.1353			

Multiple discriminant (canonical variate) analysis was used to relate the biological site groupings to the environmental data. This analysis seeks linear combinations of variates that have the greatest between-group variation relative to their within-group variation. The analysis was performed using the statistical package, SPSSX (Norusis 1985).

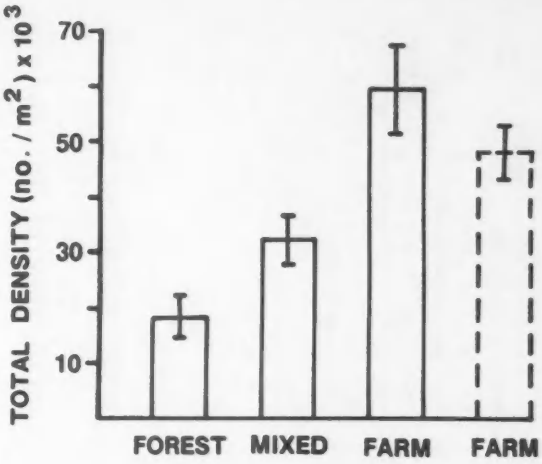

FIG. 5. Mean and standard error of benthic density at river sites for all three sampling seasons associated with the a priori land use designations. The designations were forested (Credit River sites 1,2,3,4,5), mixed (Ausable River sites 3,4,5; Maitland River sites 4,5), and farmland (Ausable River sites 1,2; Maitland River sites 1,2,3). The histogram depicted in dashed lines represents the farming sites excluding all samples from the locally enriched Maitland River site 2.

Results

Macroinvertebrate Density

Significant variability in density was evident among rivers, site locations along rivers, and seasons (Fig. 4, Table 1). Additionally, there were significant interactions among each of these three factors (Table 1). Density was lower at sites on the Credit River than for equivalent sites on the Ausable or Maitland rivers (Fig. 4). Maximum benthic density was recorded at site 2 (downstream from the town of Harriston; population 1884) on the Maitland River. Here, Oligochaeta (worms), Chironomidae (midge larvae), and Tricladida (flatworms) dominated the samples. The prevalence of midges and worms as well as the high benthic density of macroinvertebrates at site 2 reflect local nutrient enrichment from the town.

I also examined mean benthic density for sites associated with forested, farming, and mixed land use areas. Lowest and highest mean densities occurred at sites in forested and farmland areas, respectively, with sites in mixed land use areas having intermediate values (with or without the locally enriched site 2 of the Maitland River) (Fig. 5).

River Site Groupings

The cluster analysis separated the 45 collections (15 sites × 3 seasons/site) characterized by the dominant 24 taxa into three major groups (A, B, C) (Fig. 6). River sites within each group were examined for consistency with respect to sample season, site location along a river, rivers, and adjacent land use (Table 2). Consistency was defined as the maximum number of sites per cluster that fell into one category expressed as a proportion of the total number of sites in that cluster.

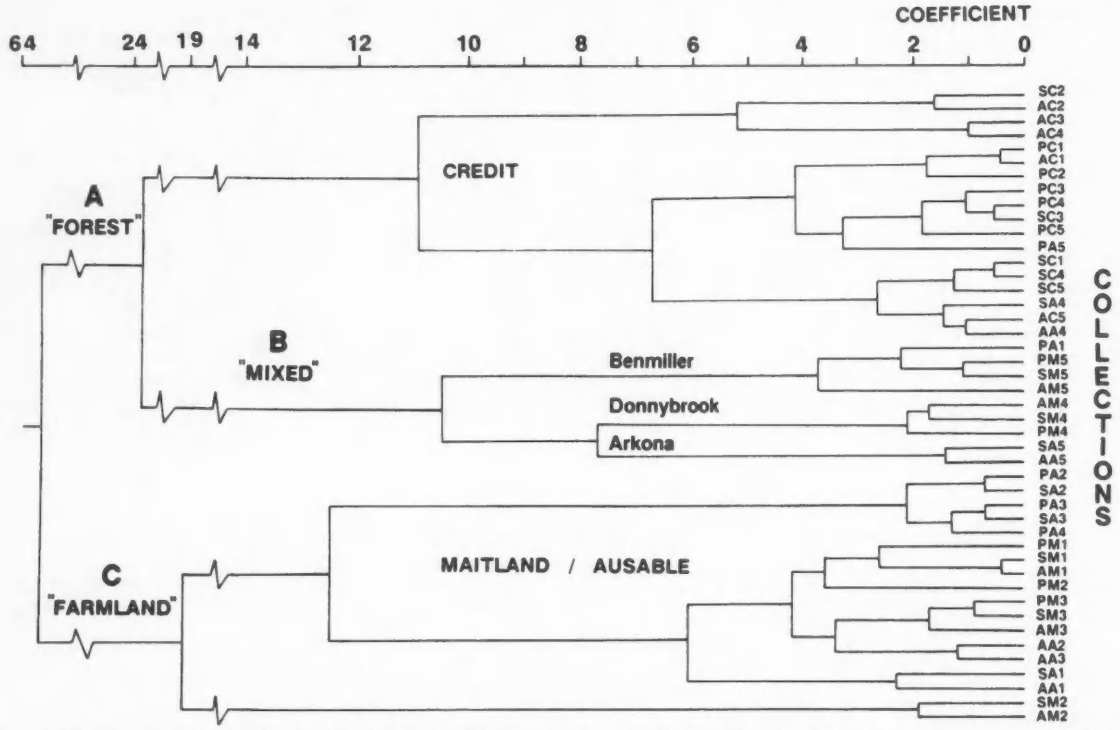

FIG. 6. Dendrogram of 45 collections (3 rivers/season × 5 sites/river × 3 seasons/site) was based on Ward's hierarchical cluster analysis for the mean ($n=5$) benthic density of the dominant taxa. Site codes include symbols for season (P, spring; S, summer; A, autumn), river (C, Credit; A, Ausable; M, Maitland) and site locations along the river from 1 (upstream) to 5 (downstream).

An equal number of sites in spring, summer, and autumn occurred in each of the three groups (Table 2). Clearly, sample sites were not grouped according to season.

Next, I examined all combinations of 5 sites sequentially to represent headwaters, mid-reach and downstream areas and compared these designations with the three groups of river sites revealed by cluster analysis. The designation of upstream (sites 1 and 2), mid-reach (sites 3 and 4), and downstream (site 5) regions provided the best fit of any arrangement of between-site locations and the three cluster groups. The highest consistency was between upstream sites and group C (61%). Poorer correspondence was noted between downstream sites and group B (55%) and between mid-reach sites and group A (44%) (Table 2).

Since each river might have a unique assemblage of macroinvertebrates, I also examined the association of the clustered groups with each of the three rivers. Although there was high consistency between Credit River sites and group A (83%), there was poorer correspondence between group B and Maitland River sites (67%) and between group C and Ausable River sites (50%).

There was a high consistency between cluster group membership and a priori land use category. Most sites in forested areas occurred in Group A (83%); sites in mixed areas corresponded well to group B (89%); and sites in farmlands were associated with Group C (78%) (Table 2). Thus, river sites were grouped more consistently according to land use than to site location along a river, specific river, or to season of collection (Fig. 6).

Macroinvertebrate Assemblages

Benthic densities of the 24 taxa were compared among the three river site groupings (A,B,C) revealed by cluster analysis. Results of 24 ANOVA tests (one per taxon) showed that mean density of Pulmonata, Hydroptilidae, Hydracarina, Simuliidae, and Elmidae did not vary significantly among clusters (Table 3). Significant differences among groups, however, were noted for other taxa. In Table 3, taxa are listed in order of magnitude of their contribution to separation of the river site groups as measured by the ratio of among to within-goup variance (F-value). Highest F-values were obtained for mayflies, Caenidae (75.17), and biting midges, Ceratopogonidae (60.03); i.e. these taxa showed the greatest variation among site groupings.

Macroinvertebrate characterization of river site groupings was based on the multiple comparison tests for each taxon in which there were significant differences in benthic density among groups (Table 3). The following macroinvertebrate assemblages characterized the river site groupings each of which was consistently associated with land use type (taxa are listed in order of the magnitude of the F-value): group A "forests" (Hydropsychidae and Leptophlebiidae), group B "mixed" (Heptageniidae, Baetidae, Psephenidae, and Tricorythidae), and group C "farmlands" (Ceratopogonidae, Oligochaeta, Corixidae, and Tricladida). Larvae of Ephemerelli-

TABLE 2. Consistency of season, site location along a river, rivers, and land use areas among the three groups of river sites (A, B, and C) generated by cluster analysis illustrated in Fig. 6.

	Season			
Grouping	Spring	Summer	Autumn	Consistency
A	6	6	6	0.33
B	3	3	3	0.33
C	6	6	6	0.33

	Site Location			
Grouping	Upstream (sites 1,2)	Midstream (sites 3, 4)	Downstream (site 5)	Consistency
A	6	8	4	0.44
B	1	3	5	0.55
C	11	7	0	0.61

	River			
Grouping	Credit	Ausable	Maitland	Consistency
A	15	3	0	0.83
B	0	3	6	0.67
C	0	9	9	0.50

	Land Use			
Grouping	Forested	Mixed	Farmland	Consistency
A	15	3	0	0.83
B	0	8	1	0.89
C	0	4	14	0.78

dae and Plecoptera were most prevalent in both "forested" and "mixed" land use groupings of river sites. Seven taxa (Caenidae, Coenagrionidae, Amphipoda, Sphaeriidae, Branchiobdellida, Chironomidae, and Hirudinea) were characteristic of those river sites in both "mixed" and "farmland" areas. Since branchiobdellids are always associated with crayfish (Pennak 1978), one would also expect crayfish occurrence in mixed and farmland river sites. No taxon had similarly high densities at river sites in both "forested" and "farmland" areas.

Environmental Variables

Values of environmental variables were examined for sample collections within each of the groupings generated using cluster analysis (Table 4). Results of one-way ANOVA tests for equality of mean values of continuous environmental variables showed that there were significant differences in AFDM ($P<0.01$), river width ($P<0.005$), and mean current velocity ($P<0.001$) among the three cluster groups that were consistently associated with land use areas. Results of the SNK multiple comparison tests indicated that AFDM at river sites in group B "mixed" and C "farmland" areas were not significantly different from one another; values of AFDM in these grouping were greater than in group A "forested" river sites. No differences in river width were discerned between Group A or C sites; widest river sites were in group B, the "mixed" land use area. Mean current velocity differed significantly among the three groups. Group C sites had the slowest-flowing water and group A river sites had the fastest-flowing water; sites in group B had intermediate values of current velocity. Since sampling depth was limited by the height of the Hess sampler (55 cm), the lack of significance in depth variation among groups should be treated with caution.

Discriminant (canonical variate) analysis was used to determine which independent environmental variables best separated river sites within the three groups revealed by cluster analysis. The first two functions were significant in discriminating among the three groups of river sites (Table 5). The overall success rate for sites correctly predicted using the set of environmental variables was 95.6%. All river sites in group A "forested" areas were correctly classified. One group B collection (PA1, spring Ausable River, site 1) was misclassified with group C. One group C collection (SM3, summer Maitland River site 3) was misclassified with group B (Fig. 6 and 7).

Figure 7 shows the separation of the three groups on the first two discriminant functions (DF1 and DF2). DF1 explained 86% of the variation among the three groups (Table 5). In function 1, mean current velocity and AFDM of benthic detritus exhibited the highest positive and negative correlations, respectively (Table 5). Mean current velocity was highest and AFDM of detritus was lowest at river sites in group A "forested" areas (Fig. 7). In contrast, mean current velocity was lowest and AFDM of detritus was highest at river sites in group C "farmland" areas. River sites in group B "mixed" areas had intermediate values of these environmental variables.

The second discriminant function explained an additional 14% of the variability among groups (Table 5). River width was the major environmental variable that accounted for the separation of sites in group B from the other two groups on the second discriminant function. Rivers were wider at sites in group B "mixed areas" than at sites in either A "forested" or C "farming" areas.

Discussion

The present study was designed to test for biome dependency by determining whether or not macroinvertebrate communities were comparable at sites among drainages within the same biome regardless of site location along a river. My study results did not support the biome dependency hypothesis. Contrary to predictions, macroinvertebrate assemblages and total density of the benthos differed among river sites in the eastern deciduous forest (EDF) biome. Moreover, community composition did not vary consistently among drainages, with site distance from river source or with season. Instead, macroinvertebrate composition and total density of the benthos was associated with the type of land use adjacent to the sample site.

Although relationships between biotic and abiotic factors along rivers have been well documented in north temperate regions (Power et al. 1988), the surrounding vegetation of the rivers that I studied did not follow the continuum model (Vannote et al. 1980) of forested headwaters (with the associated input of autumnal leaf fall) typically used to describe EDF drainage basis. The headwaters of the Ausable and Maitland rivers originated in farmlands, and flowed into forested river valleys. Deciduous forests were prevalent at all river sites sampled along the Credit.

In this study, the different types of land use were not apportioned equitably among the three drainage basins. Clearly, additional field tests are needed to examine the influence of land use "blocked" at different locations within drainage basins. Despite this limitation, my results revealed a strong correspondence between type of land use and benthic density as well as macroinvertebrate assemblages at river sites.

TABLE 3. Density of benthic invertebrates among three river site groupings (A, B, C) revealed by cluster analysis (Fig. 6). A one-way ANOVA was performed on each of 24 taxa to determine whether significant differences occurred in mean density of a taxon among groups. Data were transformed [ln(x + 1.1)] prior to analysis. The taxa are listed in order of the magnitude of their contribution to the groups as measured by the *F*-value (ratio of among-group to within-group variance). N.S. = not significant. Student–Newman–Keuls multiple comparison tests were used to compare means for each taxon. Groups linked by lines are not significantly different from one another.

			Density (mean ± SE) of macroinvertebrates (number per 0.1 m²)		
Taxon	*F*(2,42)	*P*	A "Forest" (18 sites)	B "Mixed" (9 sites)	C "Farmland" (18 sites)
Caenidae	75.17	<0.001	2.3 (0.8)	153 (38)	264 (67)
Ceratopogonidae	60.03	<0.001	0.5 (0.4)	4.8 (1.8)	53 (19)
Heptageniidae	28.21	<0.001	29 (8)	84 (13)	5.7 (1.8)
Hydropsychidae	25.37	<0.001	107 (27)	32 (20)	3.4 (1.9)
Oligochaeta	20.25	<0.001	334 (92)	1025 (414)	2179 (346)
Baetidae	19.64	<0.001	111 (30)	371 (139)	18 (9)
Psephenidae	16.08	<0.001	1.1 (0.5)	11 (3)	4.0 (1.1)
Coenagrionidae	15.86	<0.001	0.5 (0.4)	5.4 (2.5)	10 (3)
Ephemerellidae	14.79	<0.001	90 (45)	59 (37)	0.6 (0.4)
Corixidae	12.00	<0.001	0	0.8 (0.7)	29 (12)
Tricorythidae	11.38	<0.001	13 (5)	174 (71)	0.7 (0.6)
Amphipoda	11.02	<0.001	1.9 (1.0)	35 (12)	33 (8)
Plecoptera	9.43	<0.001	17 (7)	22 (19)	0.6 (0.4)
Tricladida	8.17	<0.005	6.7 (3.8)	4.9 (1.8)	133 (97)
Sphaeriidae	5.19	<0.025	1.8 (0.5)	9.0 (4.8)	39 (25)
Leptohlebiidae	5.18	<0.01	9.3 (4.1)	1.0 (0.7)	0.5 (0.4)
Branchiobdellida	5.02	<0.025	2.4 (1.6)	15 (11)	30 (11)
Chironomidae	4.71	<0.025	853 (148)	1693 (369)	1796 (351)
Hirudinea	4.65	<0.025	0.0 (0.0)	1.9 (1.2)	5.7 (3.9)
Pulmonata	3.17	N.S.	5.1 (2.6)	22 (10)	102 (67)
Hydroptilidae	2.73	N.S.	25 (8)	120 (47)	79 (57)
Hydracarina	2.21	N.S.	40 (15)	124 (72)	72 (15)
Simuliidae	1.69	N.S.	13 (9)	36 (22)	20 (19)
Elmidae	0.22	N.S.	100 (33)	110 (32)	151 (52)

TABLE 4. Comparison (mean ± SE) of the continuous environmental variables among the three river site groupings (A, B,C) generated by cluster analysis (Fig. 6). One-way ANOVA tests were used to determine significant differences in mean values of environmental variables on transformed data [ln (x + 1)] among the groups. Student–Newman–Keuls multiple comparison tests were used to compare means for each variable. Groups linked by lines are not significantly different from one another.

Variable (units)	*F* (2,42)	*P*	A "Forest" (18 sites)	B "Mixed" (9 sites)	C "Farmland" (18 sites)
Ash free dry mass (g/0.1 m²)	5.91	<0.01	1.173 ± 0.182	2.283 ± 0.580	2.421 ± 0.303
Suspended sediments (mg/L)	2.37	NS	6.8 ± 3.0	1.6 ± 0.4	3.0 ± 0.8
Minimum water temperature (°C)	0.17	NS	14.4 ± 1.4	16.0 ± 2.4	15.5 ± 1.6
Maximum water temperature (°C)	0.17	NS	19.1 ± 1.5	20.0 ± 2.7	21.0 ± 1.9
pH (pH)	1.81	NS	8.2 ± 0.07	8.3 ± 0.05	8.1 ± 0.06
Conductivity (μS/cm, 25°C)	0.38	NS	401 ± 11	423 ± 24	412 ± 12
Dissolved oxygen (mg/L)	0.70	NS	8.5 ± 0.6	8.6 ± 0.8	7.8 ± 0.8
River width (m)	6.83	<0.005	16.3 ± 1.4	47.2 ± 11.3	19.9 ± 2.8
Mean depth (cm)	0.34	NS	26.2 ± 2.3	28.8 ± 3.3	29.4 ± 2.8
Mean current velocity (m/s)	41.09	<0.001	0.514 ± 0.041	0.301 ± 0.041	0.064 ± 0.022

There is a strong association between stream characteristics and the surrounding land (Hynes 1975). Moreover, land use type is linked to the topography. For example, farms in the study area are on flat or gently rolling terrain; forests are often on hilly flood plains. Typically, more nutrients are released into receiving waters from croplands than from forested areas (Lik-

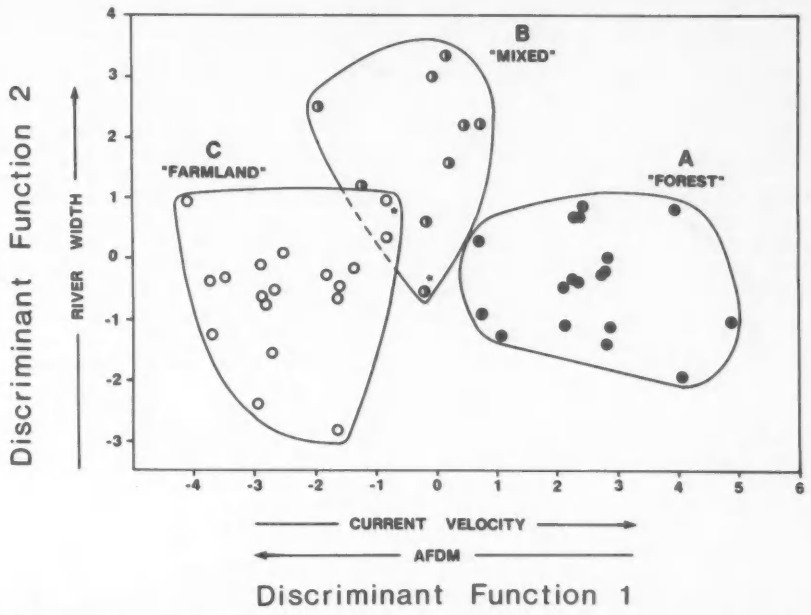

FIG. 7. Separation of river sites into group A "forested" (closed circles), group B "mixed" (half closed circles), and group C "farmland" (open circles) areas using the first two discriminant functions. Lines enclose all sites for each of the three groups. * = misclassified sites in the analysis (see text). Current velocity, ash free dry mass (AFDM), and river width are the factors most useful in delineating site groupings.

TABLE 5. Standardized canonical discriminant coefficients of environmental variables (coeff) and pooled within group correlations (corr) between variables and canonical discriminant functions.

	Function 1		Function 2	
Variable	coef	corr	coef	corr
Mean current velocity	1.025	0.69	0.087	0.20
Bank stability	0.123	0.34	0.064	0.29
Substrate	0.270	0.19	0.194	0.23
Cover	−0.116	0.12	−0.101	−0.29
Suspended sediments	0.165	0.08	−0.636	−0.30
pH	0.112	0.08	0.000	0.25
Dissolved oxygen	0.081	0.07	−0.009	0.10
Minimum water temperature	−0.776	−0.03	1.134	0.07
Maximum water temperature	0.643	−0.04	−0.952	−0.02
River width	−0.188	−0.04	0.955	0.60
Conductivity	−0.319	−0.04	0.244	0.12
Mean depth	−0.028	−0.05	−0.558	0.05
Ash free dry mass	−0.760	−0.22	0.192	0.14
X^2 (df) after each function	88.82 (26)		22.45 (12)	
Probability	<0.001		<0.03	
Variance explained (%)	86.0		14.0	

ens and Bormann 1974). Although I did not obtain direct measures for algal productivity, values of detrital AFDM (an indirect measure of production) were higher at sites in farmland and mixed areas than at sites in forested areas. River sites associated with farmland were slower-flowing, exhibited higher levels of detritus and benthic macroinvertebrate density than the faster-flowing river sites on steeper slopes of forested regions (Fig. 2 and Table 4).

Riparian vegetation controls to a large extent the rates at which sediments and nutrients enter receiving rivers (Johnson et al. 1969; Schlosser and Karr 1981). Peterjohn and Correll (1984) showed that most nutrients from agricultural lands are intercepted by riparian forests before entering receiving waters. Thus, land use within the drainage basin influences the productivity of receiving waters and the rate of transfer of materials and energy can be altered by the characteristics of streamside vegetation.

Despite the strong association between water quality of rivers and the associated landscape reported in the literature, the link between riverine fauna and the landscape is less clear. Density and biomass values of benthic macroinvertebrates are often greater in open, unshaded areas than in forested reaches with overhanging canopies, reflecting potential differences in abundance and production of benthic primary producers (Cummins 1975b). Erman et al. (1977, cited in Gregory 1983) showed a similar relationship in clearcut and forested river sites in California. There, wide (30 m) buffer strips provided shade and resulted in a decrease in benthic abundance. Other workers (Woodall and Wallace 1972; Molles 1982) have also demonstrated a correspondence between some aspect of the macroinvertebrate community and vegetation type within drainage basins. These results are consistent with the present study in which macroinvertebrate density at sites in mixed land use areas was intermediate between high levels at sites in farmlands and low levels at sites in forests (Fig. 5).

Ward (1989) conceptualized lotic systems in terms of four-dimensional axes (longitudinal, lateral, hyporheic, and temporal). In this study, macroinvertebrate assemblages corresponded more consistently with land use areas (terrestrial vegetation away from the river channel) than with either longitudinal (upstream/downstream) or temporal (seasonal) dimensions. The most consistent fit between the longitudinal

dimension and the three clusters of sites was obtained because upstream sites occurred in farmlands.

The macroinvertebrate composition at sites along rivers was independent of sample season. It is not unusual to expect that most taxonomic families would be present throughout the year within the limited geographic range examined. Coarser taxonomic designations mask the seasonal life history patterns characteristic of many species (Hynes 1970).

Because workers have had difficulty in applying empirically derived relationships developed in one river to other waterbodies (Hawkes 1975), one might consider that each river maintains a unique assemblage of invertebrates. This was not the case. Although the Credit River sites in the deciduous forest basin comprised 83% of one river grouping, the correspondence between site groupings and the Ausable and Maitland rivers was lower (Table 2, Fig. 6).

Proponents of the river continuum concept suggested that a strong link existed between riparian vegetation, the relative proportion of allochthonous and autochthonous matter, and feeding groups of stream insects along the length of rivers (Vannote et al. 1980; Cummins et al. 1984). Other workers (Winterbourn et al. 1981; Miller 1985; Hawkins 1988) have been unable to substantiate the proposed relationship between riparian vegetation and invertebrate feeding guilds. Wiggins and Mackay (1978) found that functional feeding groups of caddisflies differed between eastern deciduous forest and western montane forest biomes. The ratio of shredders to grazers in headwater regions of rivers differed between eastern deciduous (3:2) and western montane (1:1) biomes, suggesting that resource availability for grazers was greater in the west than the east (Wiggins and Mackay 1978). Thus, vegetational differences among biomes, land use areas nested within biomes, and the modifying effects of riparian vegetation are all factors to be considered in the prediction of distributional patterns of lotic macroinvertebrate assemblages.

I was able to distinguish characteristic macroinvertebrate assemblages for groups of river sites associated with different types of land use. Hydropsychid caddisflies and the leptophlebiid mayflies were most characteristic of "forested" sites with the highest mean current velocities and lowest levels of detrital AFDM in the samples. Hydropsychids are collector-filterers and/or predators; leptophlebiids are collector-gatherers or shredders. Both taxa cling to rocky substrates in lotic erosional areas (Ross 1963; Merritt and Cummins 1978). Although shredders are most often associated with forested headwater streams of EDF (Vannote et al. 1980), leptophlebiid larvae occurred along the upper 40 km of the forested drainage basin of the Credit. This suggests that surrounding vegetation may modify community composition in rivers in ways other than simply providing an energy source.

The assemblage of invertebrates at "mixed" river sites included scrapers (psephenids and baetids) and collector-gatherers (baetids, tricorythids, and heptageniids). All of these organisms cling to the substrate; baetids also may swim within the water column. All can typically be found in both lentic and lotic erosional sites; baetids and tricorythids also may occur in lotic depositional areas. These habitats were common at river sites within the mixed land use areas.

In contrast to many rivers where predators seldom account for more than 10% of the fauna at any one location and their relative abundance does not change longitudinally (Cummins 1975b; Vannote et al. 1980), the "farmland" river sites in this study were characterized by numerous predators. The characteristic fauna of farmland sites were ceratopogonids (predators or collector-gatherers), corixids (collector-gatherers and/or carnivores), oligochaetes (omnivores) and tricladids (predators). These organisms crawl (oligochaetes, tricladids) or sprawl (ceratopogonids) on riverbeds of depositional areas. Whereas ceratopogonids may swim into the water column, corixids dwell there. In this study, high nutrient levels (fertilizers) at farmland sites could account for a high turnover of species or the presence of multivoltine species that would support numerous macroinvertebrate predators. In addition, invertebrate predators are often abundant in areas devoid of fish (Macan 1977).

In summary, I have shown that there is a strong correspondence between type of land use and macroinvertebrate assemblages in rivers within a biome. Terrestrial vegetation away from the river channel (the lateral dimension) exerted a stronger influence on macroinvertebrate assemblages at river sites than factors associated with the longitudinal gradient. With changes in characteristic vegetation in the deciduous forest biome, there was a corresponding change in the lotic macroinvertebrate community. Study results were not consistent with the biome dependency model largely because the vegetation that characterized the biome had been disturbed. The "mixed" land use group of river sites (forested river valleys within farmland communities) revealed by cluster analysis was faunistically more similar to river sites in "forests" than in "farmlands." It would be useful to know how wide a buffer strip must be in relation to channel width to induce a shift in characteristic macroinvertebrate assemblages from a "mixed" to a "forested" area.

Perhaps macroinvertebrate assemblages that characterize enriched river sites in farmlands can be reset to reflect presettlement (i.e. forested) conditions by modifying the landscape adjacent to river sites. The faunal similarity between "mixed" and "forested" river groupings as well as the intermediate levels of benthic density at mixed sites between low density levels at forested sites and high levels at farmland sites suggest that such a resetting of the lotic macroinvertebrate community is possible.

Acknowledgments

I thank Terry Atchison and Mark Dunnigan for field assistance. I also thank Drs. Stuart G. Fisher, John E. Havel, Thomas R. Whittier, and particularly, Jan J. H. Ciborowski, for their constructive comments on the mansucript. Discharge data were made available from Environment Canada (Water Resources Branch). The research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada to the author.

References

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). 1985. Standard methods for the examination of water and waste water, 16th ed. American Public Health Association, New York, NY. 1268 p.

ANDREWARTHA, H. G., AND L. C. BIRCH. 1954. The distribution and abundance of animals. University of Chicago Press, Chicago, IL. 782 p.

1984. The ecological web. University of Chicago Press, Chicago, IL. 506 p.

CARTER, J. C. H., M. J. DADSWELL, J. C. ROFF, AND W. G. SPRULES. 1980. Distribution and zoogeography of planktonic crustaceans and dipterans in glaciated eastern North America. Can. J. Zool. 58: 1355–1387.

CHAPMAN, L. J., AND D. F. PUTNAM. 1984. The physiography of southern Ontario, 3rd ed. Ontario Geological Survey, Special Vol. 2, Ontario Ministry of Natural Resources. 270 p.

CLIFFORD, H. F. 1982. Life cycles of mayflies (Ephemeroptera), with special reference to voltinism. Quaest. Entomol. 18: 15–90.

CORKUM, L. D. 1989. Patterns of benthic invertebrate assemblages in rivers of northwestern North America. Freshwater Biol. 21: 191–205.

CORKUM, L. D., AND J. J. H. CIBOROWSKI. 1988. Use of alternative classifications in studying broad-scale distributional patterns of lotic invertebrates. J. N. Am. Benthol. Soc. 7: 167–179.

CUMMINS, K. W. 1975a. The ecology of running waters: theory and practice, p. 277–293. *In* Proceedings of the Sandusky River Basin Symposium. International Joint Commission, Reference Group on the Great Lakes Pollution from Land Use Activities. Tiffin, OH.

1975b. Macroinvertebrates, p. 170–198. *In* B. A. Whitton [ed.] River ecology. Blackwell Scientific Publications, Oxford, UK.

CUMMINS, K. W., G. W. MINSHALL, J. R. SEDELL, C. E. CUSHING, AND R. C. PETERSEN. 1984. Stream ecosystem theory. Verh. Int. Ver. Limnol. 22: 1818–1827.

CUMMINS, K. W., M. A. WILZBACH, D. M. GATES, J. B. PERRY, AND W. B. TALIAFERRO. 1989. Shedders and riparian vegetation. BioScience 39: 24–30.

DANKS, H. V. [ed.]. 1979. Canada and its insect fauna. Memoirs of the Entomological Society of Canada, 108. Ottawa, Ont. 573 p.

FISHER, S. G., AND G. E. LIKENS. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecol. Monogr. 43: 421–439.

GREGORY, S. V. 1983. Plant-herbivore interactions in stream systems, p. 157–189. *In* J. R. Barnes and G. W. Minshall [ed.] Stream ecology: application and testing of general ecological theory. Plenum Press, New York, NY.

HAWKES, H. A. 1975. River zonation and classification, p. 312–374. *In* B. A. Whitton [ed.] River ecology. Blackwell Scientific Press, Oxford, UK.

HAWKINS, C. P. 1988. Effects of watershed vegetation and disturbance on invertebrate community structure in western Cascade streams: implications for stream ecosystem theory. Verh. Int. Ver. Limnol. 23: 1167–1173.

HUGHES, R. M., AND J. M. OMERNIK. 1981. Use and misuse of the terms watershed and stream order, p. 320–326. *In* L. A. Krumholz [ed.] The warmwater streams symposium. Am. Fish. Soc. Allen Press Inc., Lawrence, KS.

HYNES, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Toronto, Ont. 555 p.

1975. The stream and its valley. Verh. Int. Ver. Limnol. 19: 1–15.

ILLIES, J. 1961. Versuch einer allgemeinen biozönotischen Gleiderung der Fliessgewässer. Int. Revue Ges. Hydrobiol. 46: 205–213.

JOHNSON, N. M., G. E. LIKENS, F. H. BORMANN, D. W. FISHER, AND R. S. PIERCE. 1969. A working model for the variation in stream water chemistry at the Hubbard Brook Experimental Forest, New Hampshire. Water Resour. Res. 5: 1353–1363.

LEOPOLD, L. B., M. G. WOLMAN, AND J. P. MILLER. 1964. Fluvial processes in geomorphology. W. H. Freeman and Co., San Francisco, CA. 522 p.

LIKENS, G. E., AND F. H. BORMANN. 1974. Linkages between terrestrial and aquatic ecosystems. BioScience 24: 447–456.

MACAN, T. T. 1977. The influence of predation on the composition of freshwater animal communities. Biol. Rev. Cambridge Philos. Soc. 52: 45–70.

MERRITT, R. W., AND K. W. CUMMINS, [ed.]. 1978. An introduction to the aquatic insects of North America, Kendall–Hunt, Dubuque, IA. 441 p.

MILLER, C. 1985. Correlates of habitat favourability for benthic macroinvertebrates at five stream sites in an Appalachian Mountain drainage basin U.S.A. Freshwater Biol. 15: 709–733.

MOLLES, M. C., JR. 1982. Trichopteran communities of streams associated with aspen and conifer forests: long-term structural change. Ecology 63: 1–6.

NORUSIS, M. J. 1985. SPSSX advanced statistics guide. McGraw–Hill Book Co., New York, NY. 505 p.

PENNAK, R. W. 1977. Trophic variables in Rocky Mountain trout streams. Arch. Hydrobiol. 80: 253–285.

1978. Fresh-water invertebrates of the United States, 2nd ed. John Wiley and Sons, New York, NY. 803 p.

PETERJOHN, W. T., AND D. L. CORRELL. 1984. Nutrient dynamics in an agricultural watershed: observation on the role of a riparian forest. Ecology 65: 1466–1475.

POWER, M. E., R. J. STOUT, C. E. CUSHING, P. P. HARPER, F. R. HAUER, W. J. MATTHEWS, P. B. MOYLE, B. STATZNER, AND I. R. WAIS DE BADGEN. 1988. Biotic and abiotic controls in river and stream communities. J. N. Am. Benthol. Soc. 7: 456–479.

RESH, V. H., A. V. BROWN, A. P. COVICH, M. E. GURTZ, H. W. LI, G. W. MINSHALL, S. R. REICE, A. L. SHELDON, J. B. WALLACE, AND R. C. WISSMAR. 1988. The role of disturbance in stream ecology. J. N. Am. Benthol. Soc. 7: 433–455.

ROSENBERG, D. M., [ed.] 1979. Freshwater benthic invertebrate life histories: current research and future needs. J. Fish. Res. Board Can. 36: 289–345.

ROSS, H. H. 1963. Stream communities and terrestrial biomes. Arch. Hydrobiol. 59: 235–242.

SCHLOSSER, I. J., AND J. R. KARR. 1981. Riparian vegetation and channel morphology impact on spatial patterns of water quality in agricultural watersheds. Environ. Manage. 5: 233–243.

SEIFERT, R. P. 1984. Does competition structure communities? Field studies on Neotropical *Heliconia* insect communities, p. 54–63. *In* D. R. Strong, Jr., D. Simberloff, L. G. Abele, and A. B. Thistle, [ed.]. Ecological communities: conceptual issues and the evidence. Princeton University Press, Princeton, NJ.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd ed. W. H. Freeman and Co., San Francisco, CA. 859 p.

UDVARDY, M. D. F. 1975. A classification of the biogeographical provinces of the world. International Union for Conservation of Nature and Natural Resources, Occasional Paper 18. Morges, Switzerland, 48 p.

VANNOTE, R. L., G. W. MINSHALL, K. W. CUMMINS, J. R. SEDELL, AND C. E. CUSHING. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37: 130–137.

WARD, J. V. 1989. The four-dimensional nature of lotic ecosystems. J. N. Am. Benthol. Soc. 8: 2–8.

WHITTIER, T. R., R. M. HUGHES, AND D. P. LARSEN, 1988. Correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. Can. J. Fish. Aquat. Sci. 45: 1264–1278.

WIGGINS, G. B., AND R. J. MACKAY. 1978. Some relationships between systematics and trophic ecology in Nearctic aquatic insects with special reference to Trichoptera. Ecology 59: 1211–1220.

WINTERBOURN, M. J., J. S. ROUNICK, AND B. COWIE. 1981. Are New Zealand stream ecosystems really different? N. Z. J. Mar. Freshwater Res. 16: 271–281.

WISHART, D. 1987. Clustan user manual. 4th ed. Computing Library, University of St. Andrews, Scotland. 250 p.

WOODALL, W. R., AND J. B. WALLACE. 1972. The benthic fauna in four small southern Appalachian streams. Am. Midl. Nat. 88: 393–407.

Changes in Growth and Reproduction of Harbour Porpoises, *Phocoena phocoena*, from the Bay of Fundy

Andrew J. Read and David E. Gaskin

Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1 Canada

Read, A. J., and D. E. Gaskin. 1990. Changes in growth and reproduction of harbour porpoises, *Phocoena phocoena*, from the Bay of Fundy. Can. J. Fish. Aquat. Sci. 47:2158-2163.

Changes in the growth and reproduction of harbour porpoises from the Bay of Fundy are described by comparing samples collected in 1969–73 and 1985–88. The most pronounced change was an increase in the length of calves, from 92.1 cm (SE 1.6) in 1969–73 to 108 cm (SE 1.3) in 1985–88. Females in 1985–88 attained sexual maturity at a significantly younger age (3.44 yr) and shorter length (143 cm) than females from the older sample (3.97 yr and 147 cm). These changes may be attributable to an increase in prey availability to individual porpoises. This presumed increase in prey availability may have resulted from a decrease in porpoise density caused by incidental mortality in commercial fisheries, a simultaneous increase in prey abundance, or a combination of these factors.

Les changements dans la croissance et la reproduction des marsouins communs de la baie de Fundy sont décrits par un comparaison des spécimens capturés entre 1969 et 1973 et entre 1985 et 1988. Le changement le plus marqué est une augmentation de la taille des jeunes marsouins de 92,1 cm (SE 1,6) pour la période 1969–73 à 108 cm (SE 1,3) pour la période 1985–88. Durant cette dernière, les femelles sont arrivées à leur maturité sexuelle alors qu'elles étaient notablement plus jeunes (3,44 ans) et moins grandes (143 cm) que les femelles de l'échantillon précédent (3,97 ans et 147 cm). Ces changements pourraient résulter d'un accroissement des proies accessibles à chaque marsouin. Cet accroissement de nombre de proies accessibles pourrait découler d'une diminution de la densité de marsouins provoquée par la mortalité incidente aux pêches commerciales, d'un accroissement concomitant de l'abondance des proies, ou d'une combinaison de ces facteurs.

Received January 10, 1990
Accepted June 1, 1990
(JA431)

Reçu le 10 janvier 1990
Accepté le 1 juin 1990

Many populations of dolphins and porpoises are threatened by exploitation, incidental catches, and habitat degradation (Perrin 1988). Little is known, however, about the effects of such factors on the population biology of these animals (Gaskin et al. 1984; Perrin and Reilly 1984). In particular, we have little understanding of how dolphin and porpoise populations respond to reductions in density (Perrin and Reilly 1984).

Harbour porpoises, *Phocoena phocoena* (L.), are frequently captured incidentally in commercial fisheries throughout their range (Gaskin 1984). A large number of harbour porpoises are killed in the Bay of Fundy and Gulf of Maine each year after incidental capture in groundfish gill nets (Read and Gaskin 1988). Anecdotal information suggests that these fisheries have existed in their present form for at least 15–20 yr (Read and Gaskin 1988). Estimates of current mortality levels and population size in this region are being refined, but preliminary studies indicate an annual kill of 300–800 porpoises (Read and Gaskin 1988; Polacheck 1989) and a population of between 8,000 and 15,000 (Kraus et al. 1983). The effects of this incidental mortality are unknown, but local reductions in density have been documented in some areas (Gaskin and Watson 1985).

In the Bay of Fundy, harbour porpoises feed mainly on Atlantic herring (*Clupea harengus*) (Smith and Gaskin 1974; Rechia and Read 1989). During the early 1970's, the biomass of herring in the Gulf of Maine and Bay of Fundy was greatly reduced by overfishing (Grosslein 1987). Recently, however, herring populations have substantially recovered (M. Fogarty, NMFS, Woods Hole, MA, pers. comm.). The effects of this variation in prey abundance have not been examined.

In this paper we examine changes in growth and reproduction of harbour porpoises that may be attributable to incidental mortality and/or changes in prey abundance. These changes are described by comparing growth and reproductive parameters derived from a sample collected in 1969–73 with those derived from a sample collected in 1985–88.

Methods

Harbour porpoises were captured by three methods: hunting (1969–73), incidental catches in commercial gill nets (1985–88), and incidental entrapment in herring weirs (both time periods). The numbers of porpoises captured by each method are presented in Table 1. All specimens were captured in the Bay of Fundy between July and September. Porpoises were hunted by shotgun between 1969–73, using a technique designed to sample individuals without selection for size or apparent age. Monofilament gill nets are not known to exert any sampling bias in the incidental capture of small cetaceans, although the possibility that such a bias exists has not been directly tested. Samples of harbour porpoises captured in herring weirs are known to contain disproportionate numbers of immature animals (Smith et al. 1983), so specimens from herring weirs were excluded from all comparisons that involved relative proportions of age or size classes. They were, however,

TABLE 1. Sample sizes of harbour porpoises collected in the Bay of Fundy between 1969 and 1988.

Year	Method	Male	Female	Total
1969–73	Hunted	51	47	98
	Herring Weir	15	12	27
	Total	66	59	125
1985–88	Gill Net	116	110	226
	Herring Weir	6	10	16
	Total	122	120	242

included in comparisons of growth and attainment of sexual maturity, because the bias towards immature individuals does not affect these parameters.

Standard length was measured for all specimens (American Society of Mammalogists 1961) and teeth were collected for age estimation. Mammary glands were examined for evidence of lactation and reproductive tracts of females were excised and preserved for later examination. Complete data were obtained from all porpoises collected in 1985–88, but certain observations were not recorded from some specimens collected in 1969–73.

Calves (young-of-the-year) were identified by their size, incomplete eruption of their teeth, and presence of milk in their stomachs. Verification of the identity of these individuals was provided by age estimates derived for samples from both time periods. Age estimates were obtained from counts of growth layer groups in thin, longitudinal sections of teeth (Gaskin and Blair 1977). Ages of specimens collected during 1985–88 were estimated independently by two observers, without recourse to information on the size or reproductive status of the specimen. If the two estimates for a specimen differed, the sections were re-examined by both observers. If the second reading failed to produce identical estimates, the observers examined the section together and reached consensus on the number of growth layer groups. Age estimates of porpoises collected between 1969–73 were taken from data included in Gaskin and Blair (1977). To ensure that estimates from the two time periods were similar, we examined tooth sections from a sub-sample of 41 individuals from 1969–73, and compared them to the estimates presented in Gaskin and Blair (1977). The two sets of estimates were not significantly different ($t = 0.629$, $P = 0.53$). The mean difference between the two sets of estimates was 0.05 yr (SE 0.08).

Gompertz growth curves were fitted to length-at-age data (Fitzhugh 1975). These curves have the form

$$(1) \quad SL = A(\exp(-b \exp(-k t))$$

where SL is standard length, A is the asymptotic value, b and k are constants, and t is age in years. Growth curves were fitted by least squares using a non-linear procedure (SAS Institute 1988). Residual plots were examined to ensure the models adequately fit the data.

Females were considered sexually mature if a corpus luteum or corpus albicans was present on one or both ovaries. If a corpus luteum was present, the female was assumed to be pregnant (Read 1990a). Annual pregnancy rate was estimated as the proportion of mature females possessing a corpus luteum. Variances of pregnancy rate and the proportion of mature females were calculated following Perrin et al. (1977).

Age at sexual maturity was estimated following the algorithm of DeMaster (1978). This method assumes that the proportion of sexually mature females in each age class containing both mature and immature individuals follows a monotonically increasing function. This assumption was met in the 1985–88 sample but not in the sample of females from 1969–73. To overcome this problem, the proportion of mature females in each indeterminate age class of the 1969–73 sample was smoothed using a three-parameter sigmoid function (Hohn et al. 1985). Length at sexual maturity was also estimated using the method of DeMaster (1978) and by grouping individuals into 10-cm length classes.

Results

Mean length of calves was significantly greater in 1985–88 than in 1969–73 ($t = 7.74$, $P < 0.0001$) (Table 2). There were no significant differences between the lengths of male and female calves in either the older ($t = 0.0212$, $P = 0.98$) or recent ($t = 0.416$, $P = 0.68$) samples. The mean date of collection was similar for both samples: 09 August (1969–73) and 17 August (1985–88) ($t = 1.27$, $P = 0.21$)

It was possible that the collection method affected the observed distribution of calf lengths, if small calves were captured by hunting but not by gill nets. To test this possibility, we compared the lengths of calves collected from herring weirs in the two time periods. Only two calves were captured in weirs between 1985–88, so three individuals captured between 1982–85 (A. J. Read and D. E. Gaskin, unpubl. data) were added to this sample. The mean length of calves collected from weirs between 1969–73 was 91.0 cm (SE 2.91, $n = 4$); that of calves examined between 1982–88 was 102 cm (SE 2.79, $n = 5$). Although the sample sizes were very small, the mean lengths were significantly different ($t = 2.70$, $P = 0.03$) and consistent with those reported in Table 2.

Growth curves were fitted to the length-at-age data from samples for each time period (Table 3). The increased length

TABLE 2. Comparison of mean population parameters and their 95% C.I., estimated from samples of harbour porpoises examined in 1969–73 and 1985–88 from the Bay of Fundy. Significant differences ($P < 0.05$) are indicated with asterisks. Lengths are presented in cm. Sample sizes are in parentheses.

Parameter	1969–73		1985–88	
Mean calf length	92.1 + 3.2	(20)	108 + 2.6	(21) *
Asymptotic length: Males	146 + 4.1	(56)	144 + 3.5	(121)
Asymptotic length: Females	163 + 8.4	(44)	155 + 3.5	(116)
Female age at sexual maturity	3.97 + 0.49	(37)	3.44 + 0.36	(108) *
Female length at sexual maturity	147 + 1.7	(46)	143 + 0.83	(108) *
Mean length of mature females	158 + 3.9	(19)	150 + 2.2	(50) *
Annual pregnancy rate	0.89 + 0.14	(19)	0.86 + 0.096	(50)
Proportion of females mature	0.41 + 0.14	(46)	0.46 + 0.094	(108)

TABLE 3. Parameters and their 95% C.I. estimated for Gompertz growth curves derived from samples of harbour porpoises collected in 1969–73 and 1985–88 in the Bay of Fundy.

Sample	A	b	k
1969–73: Males	146 ± 4.1	0.536 ± 0.092	0.540 ± 0.18
1985–88: Males	144 ± 3.5	0.346 ± 0.055	0.548 ± 0.17
1969–73: Females	163 ± 8.4	0.713 ± 0.12	0.476 ± 0.16
1985–88: Females	155 ± 3.5	0.475 ± 0.053	0.547 ± 0.11

of calves in 1985–88 was reflected in larger yearlings and 2-yr-old porpoises during this period than in 1969–73 (Fig. 1). Differences in the length of yearlings were also present when the comparison was restricted to porpoises collected from weirs. Insufficient numbers of 2-yr-olds were examined from weirs to allow comparison. Porpoises of both sexes and both time periods approached asymptotic lengths after 5 yr of age (Fig. 1). There were no significant differences between the asymptotic lengths of males ($t=0.740$, $P=0.49$) or females ($t=1.67$, $P=0.10$) between the two time periods (Table 2).

In 1985–88, female harbour porpoises attained sexual maturity at an earlier age ($t=11.9$, $P=0.001$) and smaller size ($t=31.1$, $P=0.001$) than those in 1969–73 (Table 2, Fig. 2). The change in length at maturity was also reflected in a lower mean length of sexually mature females in the 1985–88 sample ($t=3.65$, $P=0.0005$) (Table 2).

There were no significant differences in pregnancy rate (goodness of fit G-test, $G=0.427$, $P=0.48$) or in the proportion of females that were sexually mature ($G=1.36$, $P=0.29$) between the two time periods.

Discussion

The observed difference between calf length in the two samples reflects biological changes that have occurred between 1969–73 and 1985–88, rather than sampling biases. This conclusion is supported by the lengths of calves sampled from weirs and the consistent differences in juvenile length-at-age observed between the two samples. In addition, there was little overlap in the observed length distributions in the two samples of calves. Thus, unless the length of calves changed between the two periods, two opposite biases must be invoked to explain the observed differences: selection for large calves by gill nets and selection for small calves by hunting. Such biases appear unlikely, particularly in light of the temporal difference in calf length observed from herring weirs.

The observed increase in calf length could be caused by several factors: (i) earlier birth date and a longer period of post-

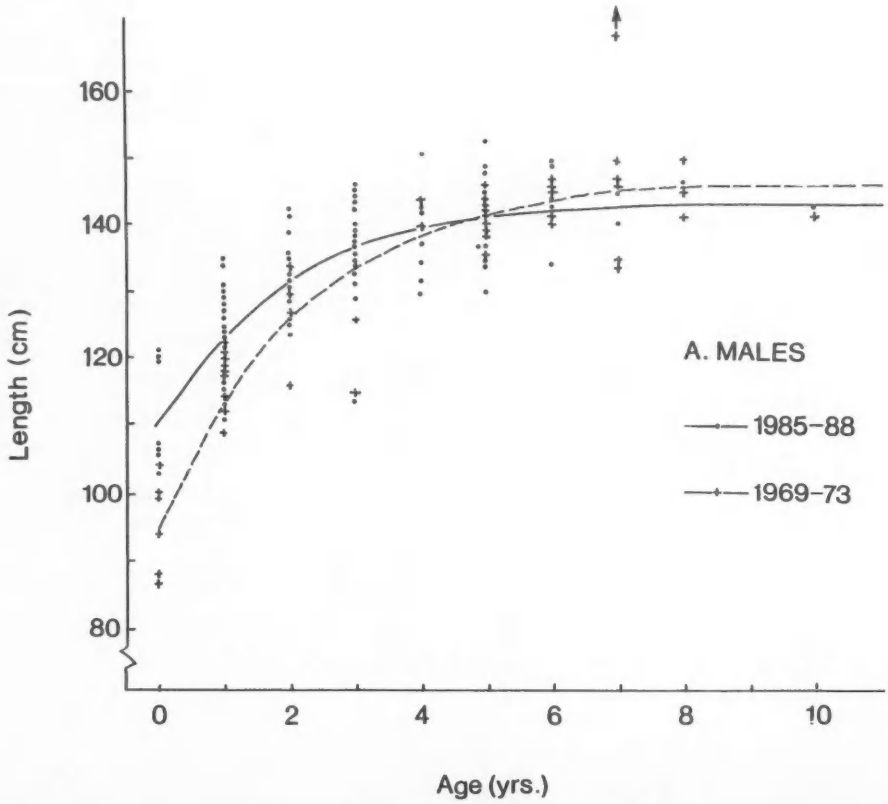

FIG. 1. Gompertz growth curves fitted to cross-sectional length-at-age data for male (A) and female (B) harbour porpoises, from the Bay of Fundy. Separate curves were fitted for samples collected in 1969–73 and 1985–88. (*Fig. 1 concluded next page*)

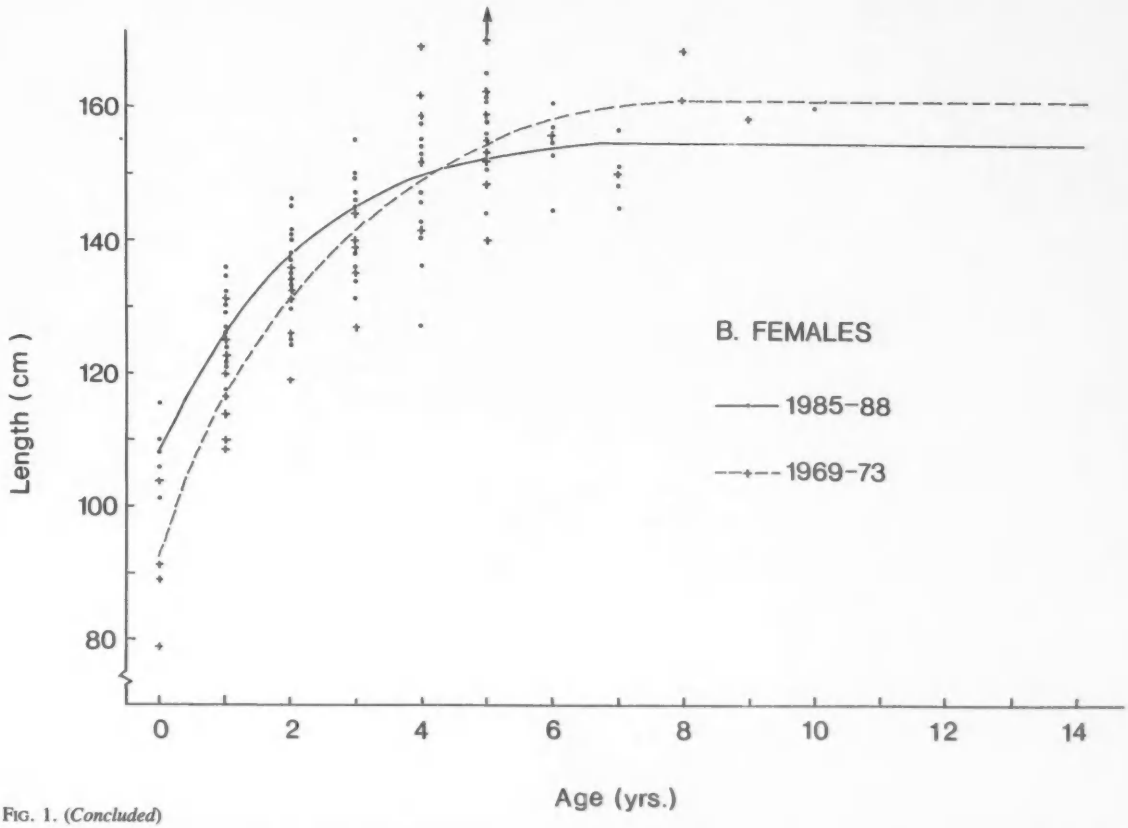

FIG. 1. (*Concluded*)

natal growth prior to sampling, (ii) increased length at birth, or (iii) increased post-natal growth rate. The date of the earliest appearance of foetuses has not changed between the two sampling periods (Read 1990a), suggesting that conception date has not changed. If calves were born earlier in 1985–88, therefore, foetal growth rates and pre-partum maternal energy investment must have been elevated accordingly. Similarly, an increase in length at birth, without a change in the timing of conception, would require higher foetal growth rates. Increased post-natal growth rates would indicate changes in post-partum maternal investment, because calves do not consume significant quantities of solid food until the autumn (Smith and Gaskin 1974; Gaskin et al. 1984) and all growth prior to this time must utilize energy obtained from lactating females. With the data at hand, it is impossible to evaluate which combination of these factors might have caused the observed increase in calf length. The available evidence, however, indicates an increase in maternal investment between 1969–73 and 1985–88. Unfortunately, no data exist to allow a direct test of this proposition. Maternal condition, as measured by blubber mass, demonstrated some annual variation between 1985 and 1988 (Read 1990b), although this was not translated into differences in calf size (Read 1990b). No comparable data exist on the body condition of harbour porpoises in 1969–73.

Temporal variation in calf size has not been investigated in other cetacean species. Studies of pinnipeds have demonstrated substantial variation in both birth size and neonatal growth. Changes in the condition of female harp seals (*Phoca groenlandica*) are reflected in changes of the size of their pups at birth; small pups are born during years when females possess low energy reserves (Stewart and Lavigne 1984; Kovacs and Lavigne 1985). Although some compensatory growth occurs in harp seal pups, differences in length at birth persist at least until weaning (Stewart and Lavigne 1984) and perhaps throughout life (Innes et al. 1981).

The increase in calf length between 1969–73 and 1985–88 was reflected by increased length in subsequent juvenile age-classes, but not by an increase in asymptotic length. It is possible that the lack of an increase in asymptotic length is due to the time lag inherent in such growth analyses. For example, a 10-yr-old porpoise sampled in 1985 was born in 1975; its final size would have been determined years before it was sampled. If changes in calf size have only recently occurred, we would not expect to see a difference in asymptotic length.

Female harbour porpoises attained sexual maturity at an earlier age in 1985–88 than in 1969–73. The functional significance of this change is that a higher proportion of 3-yr-old females was mature in the recent sample than in the earlier sample. There were no significant changes in either the proportion of mature females or pregnancy rate between the two samples. The lack of change in the proportion of mature females is somewhat surprising, given the decrease in age at sexual maturity. It is possible that the decline in age at sexual maturity has been compensated by a slight reduction in female longevity caused by mortality in gill nets.

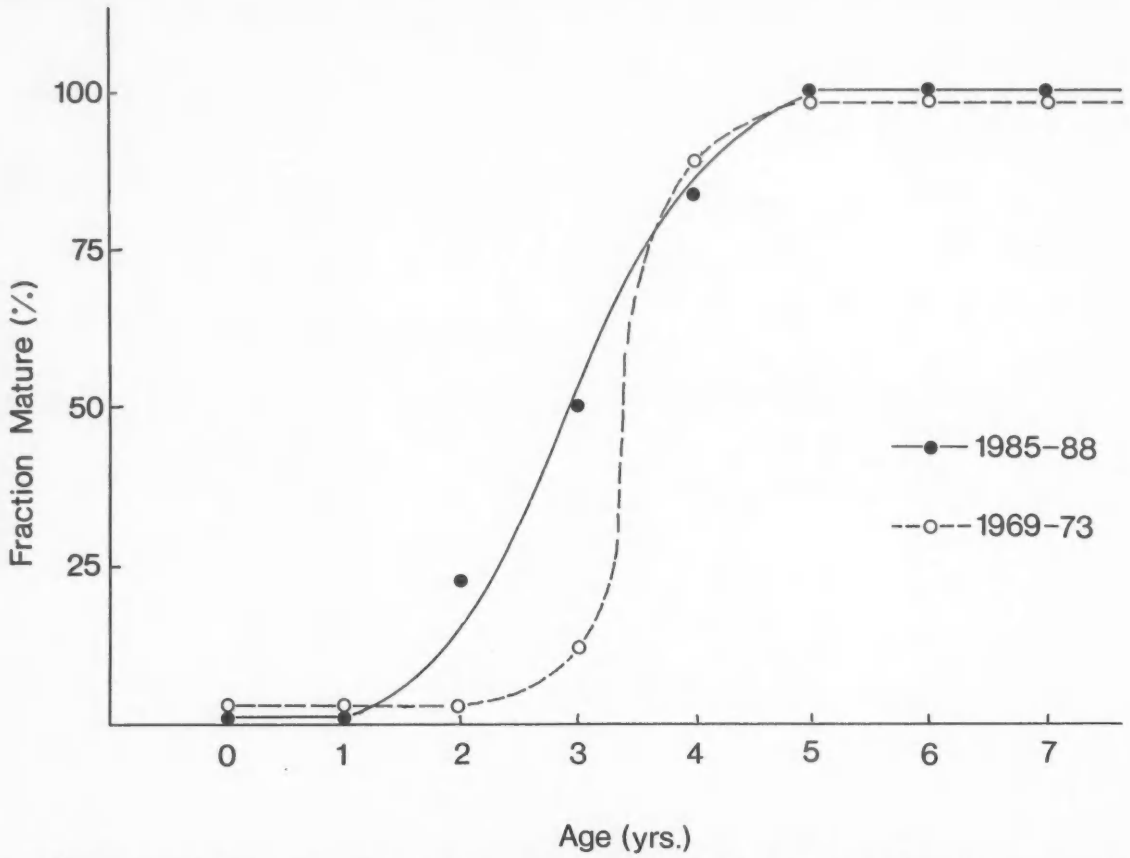

FIG. 2. Maturity ogives for female harbour porpoises from the Bay of Fundy, sampled in 1969–73 and 1985–8. Data from 1969–73 were smoothed using a three-parameter sigmoid function.

The product of pregnancy rate and the proportion of mature females represents the proportion of all females carrying a foetus. This product increased from 0.36 to 0.39 between the two sample collections. The slight increase in this parameter represents the entire reproductive response of the population to reductions in harbour porpoise density and/or increases in prey abundance. The magnitude of density—dependent reproductive change in this species may be limited by a general pattern of annual reproduction and early maturity exhibited even in the absence of significant incidental mortality.

Other studies have produced conflicting results on the effects of exploitation on the attainment of age at sexual maturity in small cetaceans. Kasuya (1985) found that mean age at sexual maturity declined from 9.7 to 7.4 yr over a 15-yr period in a heavily exploited population of striped dolphin (*Stenella attenuata*) in the western Pacific. Myrick et al. (1986) found no significant temporal change in age at sexual maturity over an 8-yr period for exploited spotted dolphins (*Stenella attenuata*) in the eastern tropical Pacific. Clausen and Andersen (1988) suggested that length at maturity decreased in populations of Danish harbour porpoises subjected to large incidental catches, but they were unable to assess changes in mean age at sexual maturity. The present results support the use of age at sexual maturity as an index of reproductive response to changes in harbour porpoise populations, although the magnitude of variation in this parameter is small.

Interpretation of the changes in growth and reproduction documented in this paper are hampered by the confounding effects of a reduction in porpoise density and a increase in prey abundance. The magnitude of incidental catches (Read and Gaskin 1988), combined with current estimates of population size (Kraus et al. 1983) suggest an annual mortality rate of between 2 and 10%. This, in combination with maximum potential population growth rates of less than 10% (Barlow 1986), suggest that the population has likely been reduced by incidental mortality. At the same time as the density of harbour porpoises was being reduced by gill nets, the abundance of their prey was increasing. During the period when the first harbour porpoise sample was collected (1969–73), herring populations in the Bay of Fundy and Gulf of Maine were at extremely low levels (Grosslein 1987). Subsequent fisheries conservation measures have allowed herring populations to recover; since 1985, herring biomass has increased to levels similar to those observed in the 1960's (M. Fogarty, NMFS, Woods Hole, MA, pers. comm).

With the data at hand, it is not possible to distinguish between the effects of reduced porpoise density and increased prey abundance. Both factors, however, would likely have allowed indi-

vidual porpoises to experience an increase in food availability. If so, this would explain how female porpoises were able to invest more energy in their calves in 1985–88 than in 1969–73.

Most studies of the effects of density reduction on cetaceans, including the present one, have focused attention on reproductive parameters (Fowler 1987). Evidence of density-dependence is more likely to be found by examining temporal variation in juvenile survival (Eberhardt and Siniff 1977). This is particularly true of species such as the harbour porpoise that possess little plasticity in their reproductive biology. In other mammals, variation in birth mass and neonatal growth are related to juvenile survival, and affect the dynamics of wild populations (Clutton–Brock et al. 1987). It is possible that the change in calf size could affect subsequent juvenile survival in harbour porpoises. There is some indirect evidence for variable juvenile survival in harbour porpoises; Mead (1979) documented an unusually large number of young harbour porpoises stranded along the North Carolina coast during the winter of 1977. Innovative research methods are required to investigate future temporal variation in survival rates of juvenile harbour porpoises.

Acknowledgements

We thank the fishermen from various ports in the Bay of Fundy who contributed porpoise carcasses. L. Murison co-ordinated the necropsy programme between 1985–88 and many other individuals, too numerous to list here, assisted with data collection between 1969 and 1988. The manuscript was improved by the comments of P. Brodie, D. DeMaster, D. Lavigne, T. Nudds, T. Woodley, and two anonymous reviewers. This research was funded by NSERC Operating Grant A5863 and contracts from Fisheries and Oceans Canada.

References

American Society of Mammalogists. 1961. Standardized methods for measuring and recording data on the smaller cetaceans. J. Mammal. 42: 471–476.

Barlow, J. 1986. Factors affecting the recovery of *Phocoena sinus*, the vaquita or Gulf of California harbor porpoise. NMFS Admin. Rep. LJ-86-37, Southwest Fisheries Center, La Jolla, CA.

Clausen, B., and S. Andersen. 1988. Evaluation of bycatch and health status of the harbour porpoise (*Phocoena phocoena*) in Danish waters. Danish Review of Game Biology 13(5). 20 p.

Clutton–Brock, T. H., M. Major, S. D. Albon, and F. E. Guiness. 1987. Early development and population dynamics in red deer. I. Density-dependent effects on juvenile survival. J. Anim. Ecol. 56: 53–67.

DeMaster, D. P. 1978. Calculation of the average age of sexual maturity in marine mammals. J. Fish. Res. Board Can. 35: 912–915.

Eberhardt, L. L., and D. B. Siniff. 1977. Population dynamics and marine mammal management policies. J. Fish. Res. Board Can. 34: 183–190.

Fitzhugh, H. A. 1975. Analysis of growth curves and strategies for altering their shape. J. Anim. Sci. 42: 1036–1051.

Fowler, C. W. 1987. A review of density dependence in populations of large mammals. Curr. Mammal. 1: 401–441.

Gaskin, D. E. 1984. The harbour porpoise *Phocoena phocoena* (L.): regional populations, status, and information on direct and indirect catches. Rep. Int. Whaling Comm. 34: 569–586.

Gaskin, D. E., and Blair. 1977. Age determination of harbour porpoise, *Phocoena phocoena* (L.), in the western North Atlantic. Can. J. Zool. 55: 18–30.

Gaskin, D. E., G. J. D. Smith, A. P. Watson, W. Y. Yasui, and D. B. Yurick. 1984. Reproduction in the porpoises (Phocoenidae): implications for management. Rep. Int. Whaling Comm. Special Issue 6: 135–148.

Gaskin, D. E., and A. P. Watson. 1985. The harbor porpoise, *Phocoena phocoena*, in Fish Harbour, New Brunswick, Canada: occupancy, distribution, and movements. Fish. Bull. 83: 427–442.

Grosslein, M. D. 1987. Synopsis of knowledge of the recruitment process for Atlantic herring (*Clupea harengus*), with special reference to Georges Bank. NAFO Science Council Studies 11: 91–108.

Hohn, A. A., S. J. Chivers, and J. Barlow. 1985. Reproductive maturity and seasonality of male spotted dolphins, *Stenella attenuata*, in the eastern tropical Pacific. Mar. Mamm. Sci. 1: 273–293.

Innes, S., R. E. A. Stewart, and D. M. Lavigne. 1981. Growth in Northwest Atlantic harp seals *Phoca groenlandica*. J. Zool. (Lond.) 194: 11–24.

Kasuya, T. 1985. Effect of exploitation on reproductive parameters of the spotted and striped dolphins off the Pacific coast of Japan. Sci. Rep. Whales Res. Inst. Tokyo 36: 107–138.

Kovacs, K. M., and D. M. Lavigne. 1985. Neonatal growth and organ allometry of northwest Atlantic harp seals *Phoca groenlandica*. Can. J. Zool. 63: 2793–2799.

Kraus, S. D., J. H. Prescott, and G. S. Stone. 1983. Harbor porpoise, *Phocoena phocoena*, in the U.S. coastal waters of the Gulf of Maine: a survey to determine seasonal distribution and abundance. Contract Rep. NA-82-FA-C-00027, Northeast Fisheries Center, Woods Hole, MA.

Mead, J. G. 1979. An analysis of cetacean strandings along the eastern coast of the United States. Biology of Marine Mammals: Insights Through Strandings, p. 54–68 *In* J. R. Geraci and D. J. St. Aubin [ed.] National Technical Information Service, Springfield, Virginia.

Myrick, A. C., Jr., A. A. Hohn, J. Barlow, and P. A. Sloan. 1986. Reproductive biology of female spotted dolphins, *Stenella attenuata*, from the eastern tropical Pacific. Fish. Bull. 84: 247–259.

Perrin, W. F. [ed.] 1988. Dolphins, porpoises, and whales. An action plan for the conservation of biological diversity: 1988–1992. IUCN, Gland, Switzerland. 30 p.

Perrin, W. F., R. B. Miller, and P. A. Sloan. 1977. Reproductive parameters of the offshore spotted dolphin, a geographical form of *Stenella attenuata*, in the eastern tropical Pacific, 1973–75. Fish. Bull. 75: 629–633.

Perrin, W. F., and S. B. Reilly. 1984. Reproductive parameters of dolphins and small whales of the family Delphinidae. Rep. Int. Whaling Comm. Special Issue 6: 97–133.

Polacheck, T. 1989. Harbor porpoises and the gillnet fishery. Oceanus 32: 63–70.

Read, A. J. 1990a. Reproductive seasonality in harbour porpoises, *Phocoena phocoena*, from the Bay of Fundy. Can. J. Zool. 68: 284–288.

1990b. Estimation of body condition in harbour porpoises, *Phocoena phocoena*. Can. J. Zool. 68: (In Press)

Read, A. J., and D. E. Gaskin. 1988. Incidental catch of harbor porpoises by gill nets. J. Wildl. Manage. 52: 517–523.

Recchia, C. A., and A. J. Read. 1989. Stomach contents of harbour porpoises, *Phocoena phocoena* (L.), from the Bay of Fundy. Can. J. Zool. 67: 2140–2146.

SAS Institute Inc. 1988. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute Inc., Cary, NC. 1028 p.

Smith, G. J. D., and D. E. Gaskin. 1974. The diet of harbour porpoises (*Phocoena phocoena* (L.)) in coastal waters of eastern Canada, with special reference to the Bay of Fundy, Can. J. Zool. 52: 777–782.

Smith, G. J. D., A. J. Read, and D. E. Gaskin. 1983. Incidental catch of harbor porpoise, *Phocoena phocoena* (L.), in herring weirs in Charlotte County, New Brunswick, Canada. Fish. Bull. 81: 660–662.

Stewart, R. E. A., and D. M. Lavigne. 1984. Energy transfer and female condition in nursing harp seals *Phoca groenlandica*. Holarct. Ecol. 7: 182–194.

Effect of Wind on the Recruitment of Canadian Arctic Cisco (*Coregonus autumnalis*) into the Central Alaskan Beaufort Sea

Robert G. Fechhelm

LGL Ecological Research Associates, Inc., 1410 Cavitt St., Bryan, TX 77801 USA

William B. Griffiths

LGL Limited, 9768 Second St., Sidney, B.C. V8L 3Y8 Canada

Fechhelm, R. G., and W. B. Griffiths. 1990. Effect of wind on the recruitment of Canadian Arctic cisco (*Coregonus autumnalis*) into the central Alaskan Beaufort Sea. Can. J. Fish. Aquat. Sci. 47: 2164–2171.

The recruitment of age 0^+ Arctic cisco (*Coregonus autumnalis*) from Canada into the Prudhoe Bay, Alaska, area for the period 1981–88 was compared with summer wind data collected at Barter Island, Alaska. Four years of poor recruitment (1981, 1982, 1984, and 1988) were characterized by winds with net easterly components ≤ 2.7 km·h^{-1} for the period 1 July–15 August. Four years in which moderate to strong recruitment occurred (1983, 1985, 1986, and 1987) were characterized by winds with net easterly components ≥ 7.5 km·h^{-1}. Results suggest that the recruitment of young-of-the-year Arctic cisco from Canada to central Alaska is strongly influenced by wind-driven currents along the Beaufort Sea coast. Recruitment may be impaired by the absence of "strong" east winds without the actual presence of prevailing west winds.

On a comparé le recrutment de ciscos arctiques (*Coregonus autumnalis*) de première année depuis le Canada jusqu'à la baie Prudhoe, en Alaska, avec les données sur les vents d'été recueillies sur l'île Barter, en Alaska, et ce, pour la période de 1981 à 1988. Quatre années de faible recrutement (1981, 1982, 1984 et 1988) se caractérisaient par des vents avec des composantes de secteur est nettes $\leq 2{,}7$ km·h^{-1} pour la période du 1er juillet au 15 août. Quatre années de recrutement moyen (1983, 1985, 1986 et 1987) se caractérisaient par des vents dont les composantes de secteur est nettes étaient $\geq 7{,}5$ km·h^{-1}. Ces résultats donnent à penser que le recrutement de ciscos arctiques de l'année depuis le Canada jusqu'à la partie centrale des côtes de l'Alaska est fortement influencé par les courants induits par les vents le long de la côte de la mer de Beaufort. Le recrutement pourrait être freiné par l'absence de vents de secteur est «forts» sans qu'il y ait pour autant de vents d'ouest dominants.

Received December 12, 1989
Accepted June 1, 1990
(JA403)

Reçu le 12 décembre 1989
Accepté le 1 juin 1990

The anadromous Arctic cisco (*Coregonus autumnalis*) is found in the coastal waters of the Beaufort Sea from Point Barrow, Alaska, to Cambridge Bay, Canada (Scott and Crossman 1973) (Fig. 1). Despite a broad coastal distribution, Arctic cisco found in Canada and Alaska have been proposed to originate from spawning stocks in Canada's Mackenzie River system (Gallaway et al. 1983). In spring, newly hatched young-of-the-year are flushed downstream to the Mackenzie River delta where they disperse both east and west along the Beaufort Sea coast. Although some Arctic cisco rear in Canada (Jones and DenBeste 1977; Byers and Kashino 1980; Hopky and Ratinski 1983; Bond 1982; Lawrence et al. 1984; LGL 1989), others disperse westward into Alaska where they take up residence in the major river deltas of the North Slope (Griffiths et al. 1983; Fawcett et al. 1986; Moulton et al. 1986a; Glass et al. 1988; Reub et al. 1988; Moulton 1989; LGL 1989). These fish remain in Alaska until the onset of sexual maturity (beginning about age 7^+), at which point the adults presumably migrate back to the Mackenzie River system to spawn (Gallaway et al. 1983).

Subsequent studies have supported the Mackenzie River origin hypothesis. Fish monitoring surveys conducted along the Alaskan and Canadian Beaufort Sea coast have tracked the westward movement of young-of-the-year Arctic cisco during the open-water summer season (Fawcett et al. 1986; Bond and Erickson 1987; Cannon et al. 1987; Moulton 1989; Reub et al. 1988). Ongoing environmental studies conducted in Prudhoe Bay, Alaska, regularly report a marked absence of older age classes of Arctic cisco — a trend which is consistent with the theory that these fish return to Canada as they reach sexual maturity (Griffiths and Gallaway 1982; Critchlow 1983; Griffiths et al. 1983; Woodward–Clyde Consultants 1983; Fawcett et al. 1986; Moulton et al. 1986a; Cannon et al. 1987; Glass et al. 1988; Reub et al. 1988; Moulton 1989; LGL 1989). The rapid disappearance of tagged Arctic cisco from a commercial fishery in the Colville River, Alaska, similarly indicates that mature fish leave the central Alaskan region (Moulton et al. 1986b). Genetic studies also support the Mackenzie River origin hypothesis, although a minority geneotype not associated with known Mackenzie stocks has also been identified (Bickham et al. 1989).

While support for the Mackenzie River origin hypothesis continues to mount, there is still a controversy over the mechanism by which young-of-the-year Arctic cisco move from Canada to Alaska. It has been proposed that these fish are transported via wind-driven coastal currents (Gallaway et al. 1983); that the process is a migration from the Mackenzie River to overwintering grounds in Alaska's Colville River (Cannon et al. 1987); and that the process is a combination of the previous two, a generalized westward migration, the extent of which is

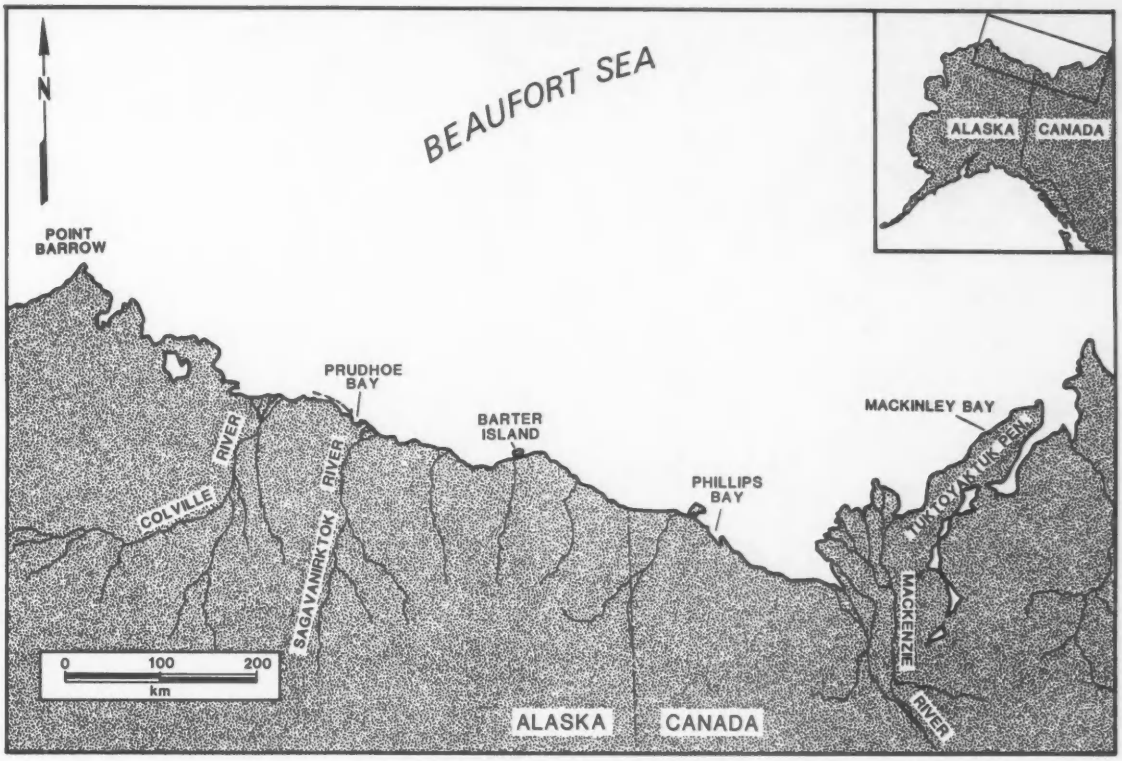

FIG. 1. Alaskan/Canadian Beaufort Sea coastline.

modified by wind-driven current regimes. The issue has reached particular prominence because the westward dispersal of young-of-the-year Arctic cisco may bring fish in close proximity to the oil fields of Prudhoe Bay, Alaska, and there is concern that petroleum production activities may negatively impact these fish. An increased understanding of the dynamics of the recruitment mechanism is necessary to accurately assess any potential or real environmental impact.

This paper analyzes the effects of coastal winds on the yearly recruitment of age 0^+ Arctic cisco into the central Alaskan Beaufort Sea. While deep offshore currents in the Beaufort Sea are governed by the Arctic Basin Gyre, nearshore flow during the ice-free summer season is primarily wind-driven and therefore variable in direction (Aagaard 1978). Prevailing winds thus determine current direction in the nearshore coastal zone. Our purpose was to determine if these summer wind patterns along the Beaufort Sea coast could be quantitatively linked to strength of the yearly recruitment into the Prudhoe Bay region.

Methods

Fish data used in our analysis were collected from 1981 to 1988 as part of summer environmental monitoring programs conducted in Prudhoe Bay, Alaska (Griffiths and Gallaway 1982; Critchlow 1983; Griffiths et al. 1983; Woodward–Clyde Consultants 1983; Biosonics Inc. 1984; Moulton et al. 1986a; Cannon et al. 1987; Glass et al. 1988; Reub et al. 1988; LGL 1989). In these studies, fyke nets — a live-capture gear — were deployed in arrays along the coast and fished daily throughout most of the open-water season (late June through mid-September). Captured fish were identified, enumerated, and released. The catch data from these fyke-net studies formed the basis of the analysis.

Wind data were collected at the National Weather Service (NEW/NOAA) meteorological station located at Barter Island, Alaska (Fig. 1). The Barter Island station is located mid-way between the Mackenzie and Colville rivers and it was felt that wind regimes would be most representative of conditions occurring along the route of transport from Canada to Alaska. Wind conditions were converted from polar coordinates (x, Ø) to linear coordinates (x, y) with a wind vector, or the resultant average of many wind vectors represented by a single point. The ordinate (x) and abscissa (y) thus represent the east/west and north/south wind components, respectively.

For each of the eight study years, average wind vector points were calculated for the period 1 July–31 August. This encompasses most of the open-water season during which movement of age 0^+ Arctic cisco from the Mackenzie River to Prudhoe Bay takes place (Fawcett et al. 1986; Bond and Erickson 1987; Cannon et al. 1987; Moulton 1989). The months of June and September were excluded from the analysis because it was felt that variable yearly ice cover during these months could bias the results — ice cover could negate the effect of winds upon currents. Wind data for the period 1 July–15 August was also analyzed since, during good recruitment years, age 0^+ Arctic cisco typically arrive in the Prudhoe Bay area by about mid-August (Cannon et al. 1987; Glass et al. 1988; Reub et al. 1988). (Wind conditions occurring after the arrival of fish in

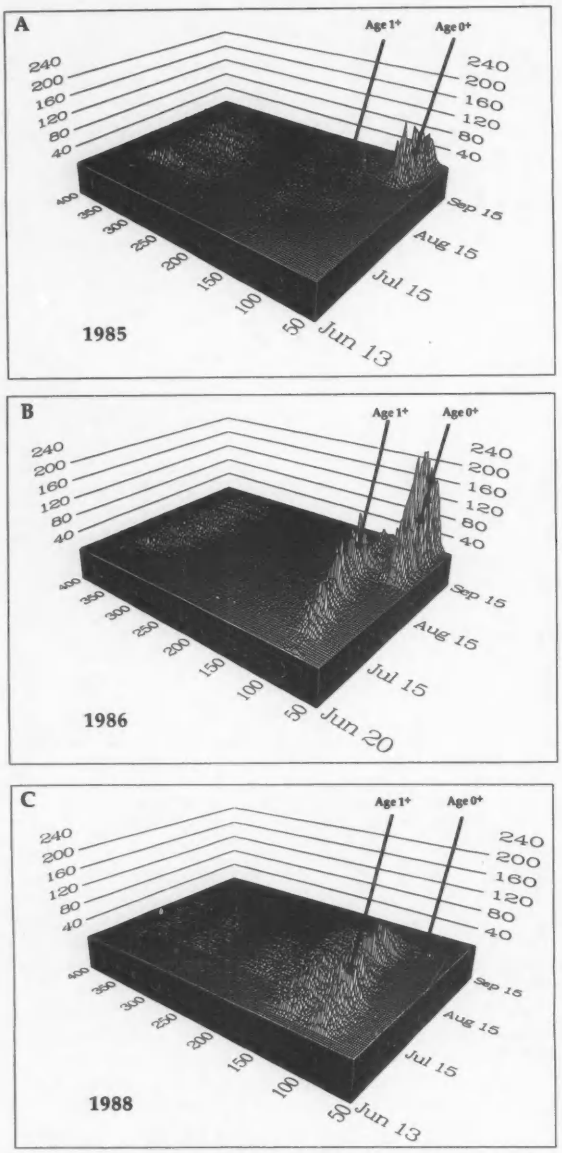

FIG. 2. Catch (number of fish) by length and date for Arctic cisco collected in the Prudhoe Bay region of Alaska during summer (A) 1985, (B) 1986, and (C) 1988. (From LGL 1989.)

the Prudhoe Bay area have no bearing on meteorological trends occurring during the actual time of transit.)

Results

Small Arctic cisco collected in Prudhoe Bay during summer comprise two discrete length cohorts which correspond to age 0^+ and age 1^+ fish (Fig. 2). These length-at-age relationships are consistent with otolith aging data obtained from the central Alaskan Beaufort Sea (Craig and Haldorson 1981; Moulton et al. 1986a; LGL 1989). Age 1^+ cisco — fish that arrived from the Mackenzie River the previous year — are present throughout most of the summer. They typically begin the summer (July) at about 70–100 mm (fork length), reaching about 130–140 mm by September. In mid-August, sudden and dramatic increases in the catch of 70–100 mm cisco denote the arrival of young-of-the-year fish from the Mackenzie River (Fig. 2).

TABLE 1. Arrival date in Prudhoe Bay area and subsequent catch rates for age 0^+ Arctic cisco by year.

Year	Arrival date	Total catch	CPUE (fish/net/day)
1982	na	0	0.0
1983	27 Aug.	2 968	24.2
1984	na	0	0.0
1985	16 Aug.	81 000[a]	144.0
1986	10 Aug.	115 000[a]	153.6
1987	13 Aug.	31 000[a]	67.3
1988	3 Sept.	180	1.1

[a]Estimated (see Methods).

Length–frequency distributions were also used to estimate recruitment success. For example, the distribution of the catch for 1986 reflected good recruitment for both 1985 (abundance of age 1^+ fish) and 1986 (a sharp increase in numbers of age 0^+ fish in late summer) (Fig. 2). Good recruitment in 1985 was confirmed by the length–frequency distribution pattern of the 1985 catch, which showed a marked increase in the catch of age 0^+ fish in mid-August (Fig. 2). The 1985 data also showed relatively low numbers of age 1^+ fish, which was consistent with the poor recruitment reported in 1984 (Moulton et al. 1986a). The 1988 catch typified a poor recruitment year in that there was no major surge of age 0^+ fish in late August and September (Fig. 2), but, the season-long predominance of age 1^+ fish was consistent with the strong recruitment observed in 1987 (Reub et al. 1988).

The catch trends outlined above were quantified in terms of catch-per-unit-effort (number of fish caught per fyke net per day) of age 0^+ fish for each summer from 1982 to 1988. Individual size cohorts were distinguished by visual inspection of yearly length–frequency distributions. In cases where there was overlap in size groups, the analytical methods described by Macdonald and Green (1985) for fitting mixtures of distributions were applied under the constraint that the means of the two cohorts were assumed to lie along a von Bertalanffy growth curve. It has been standard practice in the field to measure subsamples (100–200 fish of each species per net) when catches are extremely large. In these cases, the proportionate contribution of an individual size cohort to the subsample was applied to the total catch as an estimate of total cohort CPUE. Because young-of-the-year cisco are absent from the Prudhoe Bay area during the first half of the summer, catch rates were not calculated until the initial arrival of new recruits. This was defined as the first day in which any single fyke net caught more than two age 0^+ Arctic cisco for two consecutive days followed by a continuous and marked increase in the catch of young-of-the-year throughout the study area. This approach eliminated bias from spurious and isolated taxonomic identifications and adequately delineated major influxes of young-of-the-year fish.

From 1982 to 1988, catch-per-unit-effort of age 0^+ Arctic cisco ranged from 0.0 to 153.6 fish·net^{-1}·24 h^{-1} (Table 1). The largest catches occurred in 1983, 1985, 1986, and 1987, indicating moderate to good recruitment in those years. This

contrasted sharply with 1982, 1984, and 1988 in which catch rates were ≤ 1.1 fish·net^{-1}·24 h^{-1}. Catches were so low in 1982 and 1984 that no young-of-the-year arrival date could be assigned. Poor recruitment in 1982, 1984, and 1988 was confirmed by low numbers of age 1^+ fish caught during the following years of study: 1983 (Woodward–Clyde Consultants 1983), 1985 (Cannon et al. 1987), and 1989 (LGL 1990).

Although length measurements were not recorded regularly in 1981, we classified it as a poor recruitment year based upon interviews with field personnel who reported no presence of young-of-the-year Arctic cisco at any time during the sampling season (D. Schmidt, Dames and Moore, 5761 Silverado Way, Anchorage, Alaska 99501; R. Dillinger, LGL Ecological Research Associates, 1410 Cavitt Street, Bryan, Texas 77801; William Griffiths, LGL Limited, 9768 Second Street, Sidney, British Columbia V8L 3Y8; pers. comm.). These observations were consistent with low catches of age 1^+ Arctic cisco in 1982 (Critchlow 1983; Griffiths et al. 1983).

The plot of wind data for 1 July–31 August showed that wind regimes with the strongest easterly components occurred during the good and moderate cisco recruitment years of 1983, 1985, and 1986, while the weakest average easterly wind components occurred in the poor recruitment years of 1981, 1982, and 1984 (Fig. 3). Resultant winds for 1988 (poor recruitment) and 1987 (good recruitment) were virtually identical. When the last 2 wk in August were removed from the analysis, however, there was

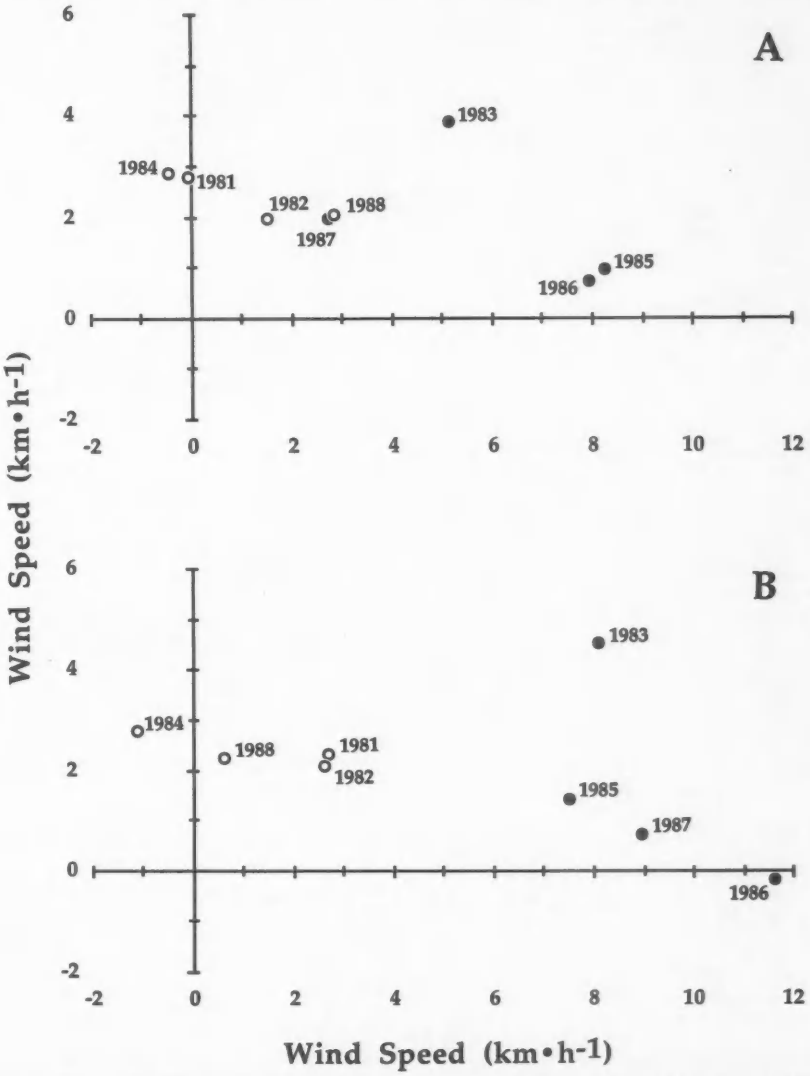

FIG. 3. Resultant winds at Barter Island, Alaska, by year for the periods (A) 1 July–31 August and (B) 1 July–15 August. The horizontal axis denotes the value of the east/west wind component; the vertical axis denotes the value of the north/south wind component. Open symbols represent poor recruitment years; closed symbols represent moderate to good recruitment years.

TABLE 2. Summary statistics for resultant east/west and north/south wind components covering the period 1 July–15 August.

Year	N	East/West (km·h^{-1}) Mean	SD	SE	North/South (km·h^{-1}) Mean	SD	SE
Moderate/good recruitment							
1983	46	8.09	13.58	2.00	4.55	4.31	0.63
1985	46	7.50	14.36	2.12	1.43	5.86	0.86
1986	46	11.64	12.86	1.87	−0.19	4.54	0.67
1987	46	8.96	18.93	2.79	0.71	6.49	0.96
Poor recruitment							
1981	46	2.68	16.48	2.43	2.34	4.13	0.61
1982	46	2.62	14.68	2.16	2.08	4.44	0.65
1984	46	−1.11	15.99	2.36	2.79	7.05	1.04
1988	46	0.63	17.31	2.55	2.24	6.92	1.02

a pronounced difference in net winds between the weak and moderate to strong recruitment years (Fig. 3). Moderate to strong recruitment occurred in all 4 yr having strong (≥7.5 km·h^{-1}) east winds while poor recruitment occurred in all 4 yr having weak (≤2.7 km·h^{-1}) east winds (Table 2). A non-parametric Mann–Whitney *U*-test indicated a significant difference (P = 0.028, two-tailed) in resultant east to west wind components based upon comparison of the four moderate to good recruitment years vs. the four bad recruitment years.

Discussion

Results support the Gallaway et al. (1983) hypothesis that the recruitment of young-of-the-year Arctic cisco from the Mackenzie River to the central Alaskan Beaufort Sea is strongly influenced by wind-driven coastal currents[1]. Data suggest that strong east winds are necessary for good recruitment and that weak east winds may be insufficient for transporting large numbers of fish into central Alaska. Recruitment may therefore be impaired without the actual presence of prevailing west winds. Results are also consistent with observed correlations between coastal wind patterns during the year of recruitment and subsequent commercial fishery catches of Arctic cisco in the Colville River (Fechhelm and Fissel 1988).

While wind-driven currents appear to play a substantial role in the dispersal of young-of-the-year Arctic cisco into the central Alaskan Beaufort Sea, other factors may also contribute to the magnitude of the yearly recruitment. For example, the age–frequency distribution for Arctic cisco collected in Alaska during 1988 (LGL 1989) revealed a marked absence of 1981, 1982, 1984, and 1988 Arctic cisco, which is consistent with poor recruitment during those years (Fig. 4). The absence of fish greater than age 9^{+} (1979 year-class) from the Alaskan length–frequency distribution presumably reflects the return of older fish to their Mackenzie River spawning grounds (Gallaway et al. 1983). Age–frequency distributions for 1988 from Canada show a more complete representation into the higher age categories. However, the Canadian data also show a weak pres-

[1]As this paper went to press, results of the 1989 summer Prudhoe Bay fish study were being finalized (LGL Alaska, Inc., 4175 Tudor Centre Drive, Anchorage, AK 99508). Data indicated poor recruitment of young-of-the-year Arctic cisco and a season of weak east winds. If poor recruitment is confirmed by a weak presence of the 1989 year class in the Prudhoe Bay area during the summer of 1990, it would represent the ninth of nine consecutive years of study in which the wind/recruitment hypothesis has held true.

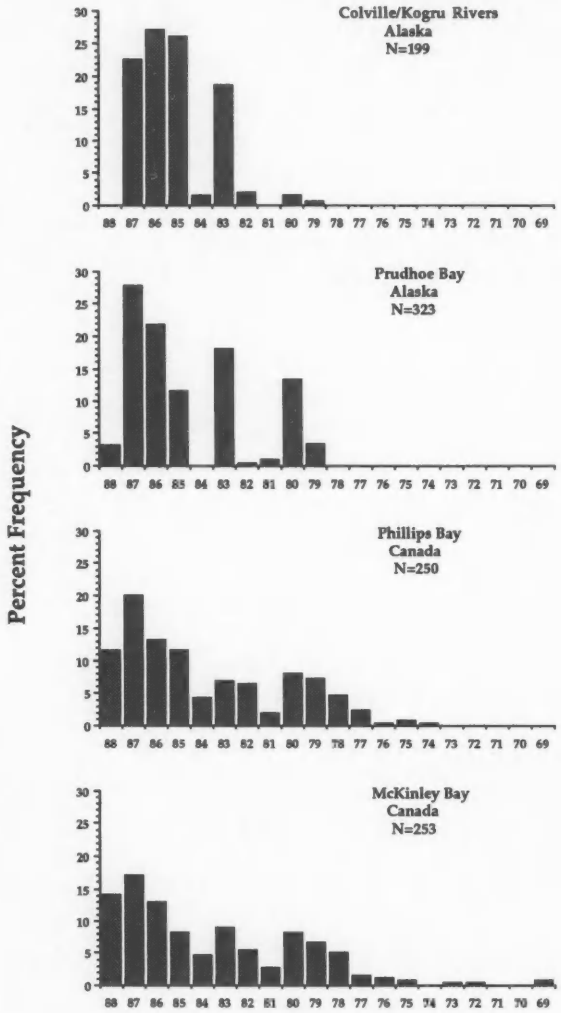

FIG. 4. Age–frequency distribution for Arctic cisco collected at four locations in the Alaskan/Canadian Beaufort Sea during summer 1988. (From LGL 1989.)

ence of the 1981 and 1984 year-classes in both McKinley Bay and Phillips Bay — two geographically distant locations (Fig. 1). The low prevalence of these cohorts in the age structure indicate that the 1981 and 1984 year-classes were weakly represented throughout the Beaufort Sea, reflecting any number of biotic or abiotic factors (e.g. poor spawning rates, low egg survival, low river flushing, or high fry mortality) that could reduce the number of young-of-the-year available for recruitment into Alaska. Low overall abundance of young-of-the-year throughout the Beaufort Sea coupled with weak east wind conditions probably accounted for the complete absence of young-of-the-year in 1981 and 1984.

The absence of the 1981, 1982, 1984, and 1988 year-classes in the age–frequency distribution of Prudhoe Bay and Colville River Arctic cisco also suggests that the en masse recruitment

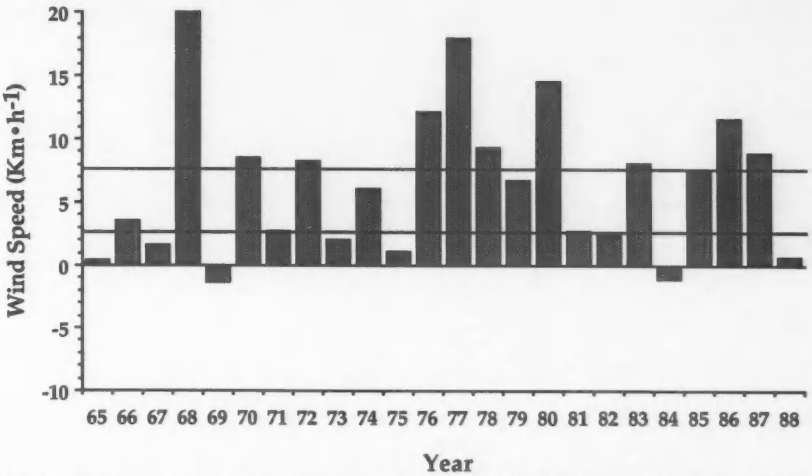

FIG. 5. Resultant east/west wind components covering the period 1 July–15 August for the years 1965–88. Positive values represent net east winds; negative values represent net west winds. Upper horizontal line denotes resultant wind components ≥ 7.5 km·h^{-1} (moderate to good recruitment) and the lower horizontal line denotes resultant wind components ≤ 2.7 km·h^{-1} (poor recruitment) Available data for 1979 covered only the period 1 July–7 August.)

of age 0^+ fish into Alaska may be limited to the year of hatching, and that, if a year-class does not reach the region in large numbers as young-of-the-year, they do not recruit as older fish. This concept is supported by the variable abundance of 1 yr olds in the Prudhoe Bay area. The four moderate to strong recruitment years of 1983, 1985, 1986, and 1987 were followed by years in which there was a marked presence of age 1^+ fish (Moulton et al. 1986a; Glass et al. 1988; Reub et al. 1988; LGL 1989). Conversely, there has been no comparable presence of age 1^+ Arctic cisco in the Prudhoe Bay area following the poor recruitment years of 1981, 1982, 1984, and 1988 (Critchlow 1983; Griffiths et al. 1983; Woodward–Clyde Consultants 1983; Cannon et al. 1987; LGL 1990). Studies have also shown that poorly recruited year-classes are weakly represented in the Colville River commercial fishery (Moulton and Field 1988; Moulton et al. 1989). This recruitment scenario could be linked to the overwintering requirements of the Arctic cisco. Strongly recruited year-classes that take up residence in the Sagavanirktok River adjacent to Prudhoe Bay appear to maintain some degree of fidelity to that drainage for the first few years of their lives (Griffiths and Gallaway 1983; Reub et al. 1988; LGL 1989). Young-of-the-year may imprint on the river in which they initially overwinter regardless of the circumstances that bring them into contact with that drainage. The stage thus appears to be "set" during the year of hatching and initial dispersal. The continual absence of specific year-classes following years of poor recruitment could also reflect the overwintering capacity of rivers lying east of the Sagavanirktok River. If these drainages do not provide adequate overwintering habitat, young-of-the-year that disperse westward into Alaska but fail to reach the Sagavanirktok River may be faced with high first year mortality.

The idea of a wind/current governed dispersal raises interesting questions with regard to the life-history pattern of the Mackenzie population and to environmental impact assessments of the Prudhoe Bay oil fields. Under the pure migration hypothesis, young-of-the-year are believed to migrate from the Prudhoe Bay to the Colville River (Cannon et al. 1987). The Colville River is the largest river in northern Alaska and may be the only Alaskan drainage with overwintering habitat extensive enough to accommodate large numbers of Arctic cisco (Adams and Cannon 1987; Schmidt et al. 1989). This extensive overwintering capacity is a principal reason why the Colville River is believed to be the destination of the young-of-the-year fish. The migration hypothesis carries with it the connotations that (1) one or more Mackenzie River stocks are genetically predisposed to migrate to the Colville River, and (2) that because it is a point-destination migration, the Colville River represents a critical overwintering habitat essential to the continued success of those stocks.

Under the pure transport hypothesis, easterly winds and associated westerly currents carry some portion of the Mackenzie River population westward with the net distance traveled a function of prevailing wind regimes. Implicit in the transport hypothesis is the concept that reaching the Colville River is a change event governed by abiotic factors and that the Colville River itself may not be a mandatory habitat required for the continued success of Canadian Arctic cisco stocks. The composite hypothesis that certain stocks may migrate in a westward direction but that the extent of this migration is modified by wind-driven currents still carries with it the idea that dispersing into central Alaska is an occasional event largely dictated by physical processes.

A major issue associated with the growth of petroleum exploration and production activities has been the construction of solid-fill gravel causeways along the Beaufort Sea coast. These structures, which extend seaward for several kilometres, are used in the recovery of offshore petroleum reserves and also serve as support facilities for land-based operations. Prudhoe Bay lies between the Mackenzie and Colville rivers, and there is concern that the causeways may disrupt the alongshore movement of young-of-the-year. If the recruitment process is a pure migration, causeway blockage could prevent fish from reaching their critical Alaskan overwintering grounds, thereby eliminat-

ing a mandatory ecological link in their life-cycle and jeopardizing the success of one or more distinct stocks. Conversely, if either the transport or composite hypotheses are correct, the Prudhoe Bay causeways would lie more or less at the periphery of a chance dispersal range and the argument that these structures jeopardize the very existence of distinct Canadian Arctic cisco stocks would be far less compelling.

We would argue that the results of this paper and that of Fechhelm and Fissel (1988) not only support the idea of either a transport or composite dispersal mechanism, but also conflict with the pure migration hypothesis on theoretical grounds. Assuming that the 1981–88 pattern of poor recruitment in four of eight years is indicative of some longer term cycle, that portion of the Mackenzie River Arctic cisco stock that disperses westward would reach their "required" overwintering habitat in the Colville River with only a 50% success rate. Historical wind patterns from 1965[2] to 1988 indicate that net east winds of ≥ 7.5 (strong), <7.5 to >2.7 (intermediate), and ≤ 2.7 $km \cdot h^{-1}$ (weak) occur approximately 46, 16, and 38% of the time, respectively (Fig. 5). From an evolutionary perspective, it would seem unlikely that an individual would gain a selective advantage in undertaking such a migration to a habitat that natural phenomena will exclude it from half of the time. Such a strategy has a great potential for disaster since over the course of several millennia, it is reasonable to expect some extended period of weak-east-wind years that would prevent several successive year-classes from reaching their "required" habitat in the Colville River. Exclusion from a mandatory link in their life cycle could eliminate these cohorts from the reproductive pool and, depending upon the numbers of fish involved, could critically jeopardize the stock. Under these circumstances, the likelihood of Arctic cisco naturally selecting the Colville River as a mandatory habitat is questionable. The fact that major westward dispersal appears to cease after the first summer and that fish do not continue westward to the Colville River in subsequent summers further counters the point–destination migration hypothesis.

To suggest that recruitment into Alaska is a dispersal phenomenon linked to prevailing meteorological trends does not imply that these fish are unimportant to Mackenzie River stocks. There are over 600 km of coastline in the Alaskan Beaufort Sea, and as discussed previously, its usefulness to Canadian Arctic cisco is relatively unknown. There is also little information regarding the proportion of Mackenzie River cisco that are transported into Alaska vs. those that remain in Canada. Arctic cisco from the Mackenzie River disperse eastward and rear along Canada's Tuktoyaktuk Peninsula (Jones and DenBeste 1977; Byers and Kashino 1980; Hopky and Ratinski 1983; Bond 1982; Lawrence et al. 1984; LGL 1989), and it is likely that the vast Mackenzie River delta itself serves as a major overwintering area. Whether the coastline of Alaska represents rearing areas of comparable worth remains to be determined.

Acknowledgements

We thank Rob Dillinger, Jean Erwin, Benny Gallaway, G. F. Hubbard, and Joe Truett for critically reviewing the manuscript. In addition, we thank G. F. Hubbard for providing the graphics. National Weather Service data were provided by the Arctic Environmental and Information Data Center at the University of Alaska, Fairbanks. Environmental monitoring programs were funded by the Duck Island Unit owners which is operated by BP Exploration (Alaska), Inc. Environmental monitoring programs from 1981 to 1987 were conducted under the joint supervision of the Alaska Department of Fish and Game, the Environmental Protection Agency, the National Marine Fisheries Service, the North Slope Borough, the U.S. Army Corp of Engineers, and the U.S. Fish and Wildlife Service. The conclusions and opinions expressed in this paper do not necessarily represent those of any of the above mentioned agencies or industries.

References

AAGAARD, K. 1978. Physical oceanography and meteorology, chap. 2, p. 56–100.; *In* Environ. Assess. Alaskan Cont. Shelf, OCS Bio. Sci. Interim Rep. BLM/NOAA OCSEAP, Boulder, CO. (Available from NOAA, Alaska Office, P.O. Box 18008, Juneau, AK.)

ADAMS, A. B., AND T. C. CANNON. 1987. Overwintering study, chap. 7. *In* Endicott Environmental Program. Rep. by Envirosphere Company to U.S. Army Corps of Engineers, Anchorage, AK, and Standard Alaska Production Company, Anchorage, AK. 33 p. (Available from Envirosphere Company, 10900 8th St., Bellevue, WA, 98004-4405).

BICKHAM, J. W., S. M. CARR, B. G. HANKS, D. W. BURTON, AND B. J. GALLAWAY. 1989. Genetic analysis of population variation in the Arctic cisco (*Coregonus autumnalis*) using electrophoretic, flow cytometric, and mitochondrial DNA restriction analyses. Biol. Pap. Univ. Alaska 23: 112–122.

BIOSONICS INC. 1984. Prudhoe Bay Waterflood Project fish monitoring program 1983, chap. 3. Rep. by Biosonics Inc. for the U.S. Army Corps of Engineers, Anchorage, AK. 161 p. (Available from BioSonics, Inc., 4520 Union Bay Pl. NE, Seattle, WA, 98105).

BOND, W. A. 1982. A study of the fishery resources of Tuktoyaktuk Harbour, southern Beaufort Sea coast, with special reference to life histories of anadromous coregonids. Can. Tech. Rep. Fish. Aquat. Sci. 1119: vii + 90 p.

BOND, W. A., AND R. N. ERICKSON. 1987. Fishery data from Phillips Bay, Yukon, 1985. Can. Data Rep. Fish. Aquat. Sci. 635: vi + 39 p.

BYERS, S. C., AND R. K. KASHINO. 1980. Survey of the fish populations in Kugmallit Bay and Tuktoyaktuk Harbour, Northwest Territories. Rep. by Dobrocky Seatech, Limited for Dome Petroleum, Limited, Victoria, B.C. 78 p.

CANNON, T. C., B. ADAMS, D. GLASS, AND T. NELSON. 1987. Fish distribution and abundance, chap. 5. *In* Endicott Environmental Program. Rep. by Envirosphere Company to U.S. Army Corps of Engineers, Anchorage, AK. and Standard Alaska Production Company, Anchorage, AK. 138 p. (Available from Envirosphere Company, 10900 8th St., Bellevue, WA, 98004-4405).

CRAIG, P. C., AND L. HALDORSON. 1981. Beaufort Sea Barrier Island Lagoon ecological process studies: Simpson Lagoon, part IV. *In* Environmental Assessment Alaskan Continental Shelf. BLM/NOAA, OCSEAP, Boulder, Colorado. 7: 384–678. (Available from NOAA, Alaska Office, P.O. Box 1808, Juneau, AK.)

CRITCHLOW, K. 1983. Prudhoe Bay Waterflood Project fish monitorong program, 1982. Rep. by Woodward–Clyde Consultants for the U.S. Army Corps of Engineers, Anchorage, AK. 327 p. (Available from Woodward–Clyde Consultants, 701 Sesame St., Anchorage, AK, 99503.)

FAWCETT, M. H., L. L. MOULTON, AND T. A. CARPENTER. 1986. Colville River fishes: 1985 biological report, chap. 2, p. 1–86. *In* Colville River fish study. Rep. by Entrix Inc. for ARCO Alaska Inc., Anchorage, AK, North Slope Borough, Anchorage, AK, and the City of Nuiqsut, AK. (Available from ARCO Alaska, Inc. P.O. Box 100560, Anchorage, AK, 99510.)

FECHHELM, R. G., AND D. B. FISSEL. 1988. Wind-aided recruitment of Canadian arctic cisco (*Coregonus autumnalis*) into Alaskan waters. Can. J. Fish. Aquat. Sci. 45: 906–910.

GALLAWAY, B. J., W. B. GRIFFITHS, P. C. CRAIG, W. J. GAZEY, AND J. W. HELMERICKS. 1983. An assesment of the Colville River delta stock of arctic cisco — migrants from Canada? Biol. Pap. Univ. Alaska 21: 4–23.

GLASS, D., C. WHITMUS, AND M. PREWITT. 1988. Fish distribution and abundance. *In* Endicott Environmental Monitoring Survey, 1986. Draft Rep. by Envirosphere Company for the U.S. Army Corps of Engineers, Anchorage, AK. 188 p. (Available from Envirosphere Company, 10900 8th St., Bellevue, WA, 98004-4405.)

GRIFFITHS, W. B., AND B. J. GALLAWAY. 1982. Prudhoe Bay Waterflood fish monitoring program 1981. Rep. by LGL Alaska Research Associates for Woodward–Clyde Consultants, Anchorage, Alaska, and the U.S. Army Corps of Engineers, Anchorage, Alaska. 143 p. (Available from LGL Alaska Research Associates, 4175 Tudor Centre Drive, Anchorage, AK, 99503.)

GRIFFITHS, W. B., D. R. SCHMIDT, B. J. GALLAWAY, R. E. DILLINGER, W. H. NEILL, W. GAZEY, AND J. S. BAKER. 1983. Fish ecology, vol. 3. *In* B. Gallaway and R. Britch [ed.] Environmental summer studies (1982) for the Endicott Development. Rep. by LGL Alaska Research Associates Inc. and Northern Technical Services for Sohio Alaska Petroleum Company, Anchorage, Alaska. 342 p. (Available from LGL Alaska Research Associates, 4175 Tudor Centre Drive, Anchorage, AK, 99503.)

HOPKY, G. E., AND R. A. RATINSKI. 1983. Relative abundance, spatial and temporal distribution, age and growth of fishes in Tuktoyaktuk Harbour, N.W.T., 28 June to 5 September, 1981. Can. Manuscr. Rep. Fish. Aquat. Sci. 1713: v + 71 p.

JONES, M. L., AND J. DENBESTE. 1977. Tuft Point and adjacent coastal areas fisheries project. Rep. by Aquatic Environments, LTD for Imperial Oil, LTD, Calgary, AB. 152 p.

LAWRENCE, M. J., G. LACHO, AND S. DAVIES. 1984. A survey of the coastal fishes of the southeastern Beaufort Sea. Can. Tech. Rep. Fish. Aquat. Sci. 1220: x + 179 p.

LGL. 1989. The 1988 Endicott Development fish monitoring program — analysis of fyke net data. Vol. 1. Synthesis. Rep. by LGL Alaska Research Associates Inc. for BP Exploration (Alaska) Inc., Anchorage, AK, and North Slope Borough, Anchorage, AK. 160 p. (Available from LGL Alaska Research Associates, 4175 Tudor Centre Drive, Anchorage, AK, 99503.)

LGL. 1990. The 1989 Endicott Development fish monitoring program — analysis of fyke net data. Draft Rep. by LGL Alaska Research Associates Inc. for BP Exploration (Alaska) Inc., Anchorage, AK, and North Slope Borough, Anchorage, AK. 144 p. (Available from LGL Alaska Research Associates, 4175 Tudor Centre Drive, Anchorage, AK, 99503.)

MACDONALD, P. D. M., AND P. E. J. GREEN. 1985. An interactive program for fitting mixtures of distributions. Ichthus Data Systems, Hamilton, Ont. 29 p.

MOULTON, L. L. 1989. Recruitment of Arctic cisco (*Coregonus autumnalis*) into the Colville delta, Alaska, in 1985. Biol. Pap. Univ. Alaska 24: 107–111.

MOULTON, L. L., AND L. J. FIELD. 1988. Assessment of the Colville River fall fishery 1985–1987. Rep. by Environmental Sciences and Engineering Inc. for ARCO Alaska Inc., Anchorage, AK, North Slope Borough, Anchorage, AK, and the City of Nuiqsut, AK. 42 p. (Available from ARCO Alaska, Inc. P.O. Box 100560, Anchorage, AK, 99510).

MOULTON, L. L., L. J. FIELD, AND S. BROTHERTON 1986b. Assessment of the Colville River fishery in 1985, chap. 3, p. 1–72. *In* Colville River fish study. Rep. by Entrix Inc. for ARCO Alaska Inc., Anchorage, AK, North Slope Borough, Anchorage, AK, and the City of Nuiqsut, AK. (Available from ARCO Alaska, Inc. P.O. Box 100560, Anchorage, AK, 99510.)

MOULTON, L. L., L. J. FIELD, AND R. KOLVALSKY. 1989. The 1988 fall gill net fisheries for ciscoes in the Colville River, Alaska, chap. 2. *In* The 1988 Endicott development fish monitoring program: a monograph of project papers, 1988. Rep. by LGL Alaska Research Associates for BP Exploration (Alaska) Inc., Anchorage, AK. 38 p. (Available from LGL Alaska Research Associates, 4175 Tudor Centre Drive, Anchorage, AK, 99503.)

MOULTON, L., B. GALLAWAY, M. FAWCETT, W. GRIFFITHS, K. CRITCHLOW, R. FECHHELM, D. SCHMIDT, AND J. BAKER. 1986a. 1984 Central Beaufort Sea fish study. Waterflood Monitoring Program Fish Study. Rep. by Entrix Inc., LGL Ecological Research Associates Inc., and Woodward–Clyde Consultants for Envirosphere Co., Anchorage, AK, and the U.S. Army Corps of Engineers, Anchorage, AK, 300 p. (Available from Woodward–Clyde Consultants, 701 Sesame St., Anchorage, AK, 99503.)

REUB, G. S., J. D. DURST, AND D. GLASS. 1988. Fish distribution and abundance. *In* 1987 Endicott Environmental Monitoring Program. Draft Rep. by Envirosphere Company to U.S. Army Corps of Engineers, Anchorage AK, and Standard Alaska Production Company, Anchorage, AK. 47 p. (Available from Envirosphere Company, 10900 8th St., Bellevue, WA, 98004-4405.)

SCHMIDT, D. R., W. B. GRIFFITHS, AND L. R. MARTIN. 1989. Overwintering biology of anadromous fish in the Sagavanirktok River delta, Alaska. Biol. Pap. Univ. Alaska 24: 55–74.

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

WOODWARD–CLYDE CONSULTANTS. 1983. Lisburne Development Area: 1983 Environmental Studies. Rep. for ARCO Alaska Inc., Anchorage, AK. 722 p. (Available from Woodward–Clyde Consultants, 701 Sesame St., Anchorage, AK, 99503.)

Variability in Agonistic Behaviour and Salinity Tolerance between and within Two Populations of Juvenile Chinook Salmon, *Oncorhynchus tshawytscha*, with Contrasting Life Histories

Eric B. Taylor[1]

Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 1W5 Canada

Taylor, E. B. 1990. Variability in agonistic behaviour and salinity tolerance between and within two populations of juvenile chinook salmon, *Oncorhynchus tshawytscha*, with contrasting life histories. Can. J. Fish. Aquat. Sci. 47: 2172–2180.

Agonistic behaviour and salinity tolerance were investigated in eight families of chinook salmon, *Oncorhynchus tshawytscha*, from two populations in the Nanaimo River, British Columbia. From emergence to 3 mo, there were small differences in agonistic behaviour among families within populations, but "First Lake" chinook had strikingly higher levels of agonistic behaviour than salmon from the "lower Nanaimo" population. Lower Nanaimo chinook were larger, lost less weight, and had higher survival after 24 h in 20 ppt and 30 ppt seawater than First Lake salmon. Within populations, families of larger than average size tended to perform better in seawater than families of smaller size. At a common body size, salinity tolerance was similar in salmon from the two populations, but significant differences among families within populations suggest that families may differ in factors other than size that influence salinity tolerance. It was concluded that the greater agonistic behaviour of First Lake chinook has a genetic basic and is related to their longer period of territorial stream residence before seaward migration. By contrast, the two populations exhibited only small differences in salinity tolerance, but significant interfamily variation illustrates the importance of considering family effects in studies of interpopulation differentiation.

Des études ont été effectuées sur le comportement agonistique et la tolérance à la salinité de huit familles de saumon chinook, *Oncorhynchus tshawytscha*, provenant de deux populations de la rivière Nanaimo, en Colombie-Britannique. On a relevé de petites différences dans le comportement agonistique entre les familles au sein des populations depuis le stade de l'émergence jusqu'à l'âge de 3 mo mais les chinooks de «First Lake» manifestaient un comportement agonistique nettement plus prononcé que les saumons de la population de la «basse Nanaimo». Ceux-ci étaient plus gros, perdaient moins de poids et survivaient en plus grand nombre au bout de 24 h dans des eaux de mer à 20 ppt et à 30 ppt que les saumons de First Lake. Au sein des populations, les familles de taille moyenne supérieure s'adaptaient en général mieux à l'eau de mer que les familles de plus petite taille. Pour une taille commune, la tolérance à la salinité était similaire chez les saumons des deux populations mais des différences notables entre les familles des mêmes populations donnent à penser que les familles peuvent présenter des différences dans d'autres facteurs que la taille et que ceux-ci peuvent avoir des répercussions du point de vue de la tolérance à la salinité. On a conclu que le comportement plus fortement agonistique des saumons chinook de First Lake avait des fondements génétiques et tenait au fait qu'ils restent plus longtemps dans des parties territorialisées des cours d'eau. Par contre, les deux populations ne manifestaient que de légères différences dans la tolérance à la salinité mais la variation notable que l'on a relevé entre les familles montre bien à quel point il est important de tenir compte des effets propres aux familles dans les études de la différenciation entre les populations.

Received November 20, 1989
Accepted May 15, 1990
(JA364)

Reçu le 20 novembre 1989
Accepté le 15 mai 1990

Species of salmon, trout, and charr (Salmonidae) have provided rich material with which to study interpopulation variability in phenotype and its potential adaptive significance (Ricker 1972; Bams 1976; Riddell and Leggett 1981; Healey and Heard 1984; Taylor and McPhail 1985; Titus and Mosegaard 1989). Behavioural variability has been the focus of many such studies and selection associated with local environmental differences has been proposed to account for inherited differences between populations (Raleigh 1971; Quinn 1985; Rosenau and McPhail 1987; Taylor 1990).

Inherited variation in behaviour, morphology, and growth rate among populations of juvenile chinook salmon (*Oncorhynchus tshawytscha*) was proposed to result from selection associated with differences among populations in the duration of freshwater residence before seaward migration (Taylor 1988, 1990). Salmon from populations that reside and feed in streams for a year or more before smoltification ("stream-type" chinook) had higher levels of agonistic behaviour, had more pronounced seasonal changes in rheotaxis, but grew more slowly than fish from populations that migrate seaward as newly-emerged salmon or after only 2–3 mo in freshwater ("ocean-type" chinook). These studies, however, were of progeny from multiple-parent, pooled gamete crosses and thus could not assess interfamily differences in phenotype within populations, yet consideration of interfamily variability can be important for several reasons.

[1]Present address: Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1 Canada.

First, recent investigations of developmental biology (Beacham and Murray 1986a, 1986b) and growth and survival in salmonids (Withler et al. 1987), have demonstrated that differences among families within populations may equal or exceed variability between populations. Consequently, interfamily differences can have important implications in studies of interpopulation variability involving breeding experiments, particularly if the number of parents sampled is small relative to total population size. In such cases, consideration of family variability provides a more rigorous test of differences between populations because treatment effects are tested against variation associated with the number of parents sampled, not their progeny. Second, partitioning phenotypic variation into that among families within populations and that between populations provides a more thorough understanding of the extent, distribution, and causes of phenotypic variability, knowledge that is essential to the management and preservation of genetic resources of fish (Allendorf and Ryman 1987; Beacham 1988). Third, in the case of chinook salmon, studying variation among families provides an opportunity to test more directly the association between juvenile phenotype and life history by examining the characteristics of progeny produced by parents of known life history.

In the present study, I examined agonistic behaviour in families of juvenile chinook originating from two populations in the Nanaimo River which differ in freshwater life history. In addition, I examined variation in salinity tolerance since it is an important component of the transition from freshwater to estuarine/oceanic habitats and its ontogeny should vary between populations that migrate to sea at different ages. Specifically, I made two predictions. First, if selection associated with distinct life histories is responsible for promoting variability between Nanaimo River populations, then both for agonistic behaviour and salinity tolerance, variation among families within populations would be minor relative to variability between populations. Second, I predicted that the progeny of adult salmon that, as juveniles, had spent extended periods feeding in freshwater before seaward migration would have higher levels of agonistic behaviour, but lower salinity tolerance, compared to the progeny of parents that had migrated to sea soon after emergence.

Materials and Methods

Experimental Fish

Fish used in the experiments were progeny from single male × female crosses made with adult salmon from the "lower" (migrate to estuary mostly as newly-emerged salmon) and "First Lake" (migrate mostly at 2–3 mo of age) spawning populations in the Nanaimo River (see Carl and Healey 1984). A third population, which is located in the headwaters of the Nanaimo River below Fourth Lake and produces a high proportion of yearling smolts, was not sampled due to low population size in 1988. Eight full sib family matings were completed at the Nanaimo River Salmon Enhancement Facility in October 1988; three families of First Lake salmon and five families from the lower Nanaimo. Zygotes (800 per family) were disinfected with Ovadine and then placed, by family, in adjacent sections of a single incubation tray at 10–11°C at the hatchery until the "eyed" embryo stage (about 1 mo). At the eyed stage, the embryos were transferred to the University of British Columbia to complete incubation at 6°C.

The freshwater life history of the parents used in the crosses was assessed from a sample of five scales from each parent. Scales were removed from between the posterior margin of the dorsal fin and the lateral line scale row and standard length (SL) was measured. Scale impressions were made following the methods of Clutter and Whitesel (1956) and photographic prints of each impression were made at 34× under a 3M microfiche reader-printer. Freshwater circulus counts were made at 100× with the assistance of a technician from the Scale Morphology Lab of the Canadian Department of Fisheries and Oceans (Vancouver, B.C.). An index of the duration of freshwater residence before seaward migration was obtained by counting the number of freshwater circuli; fish with more freshwater circuli were assumed to have spent more time feeding in freshwater (Schluchter and Lichatowich 1977).

Upon emergence (when the young salmon have nearly absorbed their yolk reserves and begin exogenous feeding) the salmon were assigned randomly by family to one of eight 94 L rectangular rearing troughs where they were maintained under a natural photoperiod and water temperature regime. Here the salmon were fed three times per day with commercial fish food as described by Taylor (1990). Size variation at emergence was determined by taking wet weights (to 0.01 g) and standard and fork lengths (0.5 mm) for a sample of 30 individuals per family.

Variation in Agonistic Behaviour

Agonistic behaviour of juvenile chinook salmon from the eight families was studied in three stream tanks. Each tank (1.4 × 0.9 × 0.3 m) was divided into two parallel channels (1.0 × 0.35 × 0.3 m) which were separated from each other by a central compartment (1.0 × 0.25 × 0.3 m). One side of each stream channel was formed by 0.5 cm thick Plexiglas for viewing. Each stream channel was isolated from the surrounding area by stainless steel wire mesh barriers (0.3 cm mesh at emergence and 1 mo, 0.65 cm mesh at 2 and 3 mo of age) at the upstream and downstream ends. Environmental conditions in each channel ($N = 6$, 3 stream tanks × 2 channels in each) were identical; 10–11°C, natural photoperiod, "pea" gravel and small stone (3–5 cm diameter) substrate as described by Taylor (1990). A submersible pump generated water velocities of 5–6 $cm \cdot s^{-1}$ for tests at emergence and 1 mo, and 10 $cm \cdot s^{-1}$ for tests at 2 and 3 mo. Higher water velocity was required to ensure upstream orientation of the older (and larger) fish at 2 and 3 mo. The observer was visually isolated from each channel by black plastic sheeting with observation holes.

To encompass the different ages at seaward migration of Nanaimo River chinook, I examined agonistic behaviour of salmon at emergence, 1, 2, and 3 mo. Differences in spawning time (1–2 wk) and developmental rate among families permitted the testing of each family within 1 wk of the stated test ages. At each age, I took two or three samples of ten fish from each family and assigned each sample to a separate stream channel 48 h before the start of observations. For 5 min, twice daily for 5 consecutive d, I then recorded the frequency and duration of four agonistic behavioural acts ("approach," "nip," "charge," and "lateral display") and two non-agonistic ("forage" and "yawn"). Agonistic action patterns were as defined by Taylor and Larkin (1986) except for "lateral display" which included "lateral" and "wigwag" fin displays. "Forage" was defined as any swimming movement covering at least two bodylengths followed by attempted ingestion of drifting objects, and "yawn" consisted of an exaggerated opening of the mouth

by a fish accompanied by pronounced flaring of the gills and terminated by a rapid closing of the mouth.

The salmon were fed through tubes located at the upstream end of each channel after each observation period (1000 and 1700) and at mid-day at about 5% bodyweight·d^{-1}. Live *Artemia* nauplii were fed to newly-emerged and 1 mo old fish, thereafter frozen *Chiromomus* larvae were used. The salmon averaged 0.48, 1.1, 2.1, and 3.5 g at emergence, 1, 2, and 3 mo.

Variation in Salinity Tolerance

Variation in tolerance to seawater was investigated using "seawater challenge" tests (Blackburn and Clarke 1987). Weight loss from dehydration at 20 ppt seawater and mortality after transfer to 30 ppt were recorded for separate groups of fish from each family. A salinity of 20 ppt is common for the Nanaimo River estuary (Healey 1980) and 30 ppt is representative of nearshore marine conditions and is commonly used in seawater challenge tests. Filtered seawater from Burrard Inlet was standardized to 30 ppt by the addition of non-iodized salt and 20 ppt seawater was prepared by dilution with dechlorinated tap water. Salinities were determined using a hydrometer.

"Static" 24 h seawater challenge tests (Blackburn and Clarke 1987) were run in five 75 L plastic pails in a controlled environment chamber (10°C). For each test, three pails were randomly assigned as experimental (containing saline water) and the other two as freshwater controls.

Tests were run on the progeny from each family at emergence, 1, 2, and 3 mo of age. Twenty-four h before a test, 50 fish from the family being tested were isolated and deprived of food. They were then separated into five groups of ten fish, blotted on moist paper towel, and weighed individually to the nearest 0.001 g. Each group of 10 was then returned to freshwater to recover from handling. After 1 h, each group of fish was placed in one of the five pails and left for 24 h. Air bubblers in each pail provided aeration. After 24 h, the survivors in each pail were enumerated, reweighed, and preserved in 10% formalin. Percentage weight loss for each group was adjusted by subtraction of the percentage weight loss of the freshwater controls.

Statistical Analysis

Data on behaviour durations were summarized by principal components analysis (PCA) using MIDAS (Fox and Guire 1976). This procedure produced measures of agonistic behaviour consisting of one or more independent behavioural vectors as an efficient alternative to examining behavioural variation character-by-character (cf. Huntingford 1976; Nishikawa 1985). Subsequently, the derived behavioural components were multiplied through the original data to produce principal component scores for each observation period. A mean PC score was then calculated for each family at each age over all repeated measures (observation periods and days). These scores were analysed by factorial, nested analysis of variance; age and population were the main effects and family was nested within population (lower Nanaimo and First Lake Nanaimo). The data on percentage survival and weight loss in seawater were arcsin square root transformed prior to analysis and then subjected to factorial, nested analysis of covariance with ln standard length as the covariate. Since salinity tolerance is size-dependent (e.g. Parry 1960; Wagner et al. 1969; McCormick and Naiman 1984), analysis of covariance was used to adjust for size differences among families and ages and to test for size-independent variation in salinity tolerance. In all tests, homogeneity of sample variances was tested with Bartlett's test (Zar 1984) and statistical significance was declared at $P \leq 0.05$.

Results

Adult Salmon Freshwater Rearing Life History

The number of freshwater circuli in the scales of adult salmon from the Nanaimo River used to make family crosses varied from seven to 21 (Table 1). First Lake family 3 had the highest mean parental circuli count (17.5); the female had 21 circuli (with a distinct freshwater annulus) and the male had 14. Lower Nanaimo family 5 had the lowest mean parental circuli count (7.5). Adults tended to be larger if from the lower Nanaimo and they tended to produce the larger young at emergence (0.46 ± 0.07 vs. 0.39 ± 0.05 g, $P > 0.05$, df = 1,6, Table 1), but differences among families within the two populations were more notable ($P < 0.0001$, df = 6,232, Table 1).

Variation in Agonistic Behaviour

In the stream tanks, salmon from all families had similar spatial distributions. Typically, 2–4 individuals occupied the mid-water, upstream portions of each tank section. Although dominance status among individuals was not experimentally determined, a single fish appeared to be "dominant" during each replicate. Such individuals invariably occupied, and vigorously defended, a position nearest the point where food was introduced. The other one to three fish found in the upstream sections ("subdominants") usually held positions just downstream and to the sides of the dominant individual. In all families, the remaining fish in each replicate ("submissives") were positioned along the sides and/or in the downstream half of the tank sections. Most of the behavioural acts involved interaction

TABLE 1. Parental standard lengths (SL, cm) and number of freshwater circuli (FWC), and newly-emerged fry wet weights (mean ± SE in g, $N = 30$) for chinook salmon from the Nanaimo River.

Family	Female SL	Male SL	Female FWC	Male FWC	Mean FWC	Fry weight
First Lake 1	75	62	12	8	10	0.42 (0.02)
First Lake 2	76	77	10	8	9	0.43 (0.03)
First Lake 3	74	70	21	14	17.5	0.34 (0.03)
Lower Nanaimo 1	73	72	10	10	10	0.37 (0.03)
Lower Nanaimo 2	75	80	9	10	9.5	0.46 (0.03)
Lower Nanaimo 3	81	84	8	11	9.5	0.43 (0.03)
Lower Nanaimo 4	82	83	7	9	8	0.57 (0.02)
Lower Nanaimo 5	83	83	8	7	7.5	0.50 (0.03)

TABLE 2. Behaviour coefficients derived from a principal components analysis of agonistic behaviour durations of juvenile chinook salmon observed during stream tank interactions.

Behaviour	Component 1	Axis 2
Approach	0.065	0.867
Lateral display	0.987	−0.123
Charge	0.094	0.221
Nip	0.106	0.425
Forage	−0.001	0.004
Yawn	−0.002	0.055
Total variance (%)	94.1	97.3

among the dominant and subdominant fish or between these fish and submissive individuals that ventured from their positions at the downstream or side portions of the tanks into "territories" of the dominant and subdominant fish. Relatively little interaction was observed among submissive individuals.

Variation in behaviour among the eight families was summarized by two principal components encompassing 97% of the total variance (Table 2). Agonistic behavioural acts dominated both axes while "yawn" and "forage" had consistently low component loadings (Table 2). The two components, however, summarized different aspects of agonistic behaviour. Principal component 1 was dominated by "lateral display" behaviour while PC2 summarized behaviour largely in terms of overt behavioural acts, i.e. "approach," "nip," and to a lesser extent, "charge" (Table 2).

Along the first component axis (Fig. 1a) First Lake salmon had strikingly higher levels of agonistic behaviour than their lower Nanaimo counterparts over all ages combined (64% of total variability, $P=0.0002$, df = 1,6). By contrast, while significant, differences among families nested within populations accounted for only about 5% of the total variability in agonistic behaviour, ($P=0.05$, df = 6,45, Table 3). Age of the salmon was also a significant contributor to variation in agonistic behaviour along PC1 (Fig. 1, 4% of total variability, $P=0.05$, df = 3,18); juvenile salmon interacted most vigourously at 2 mo with a significant decline at 3 mo. No significant interactions were evident among the various factors (all $P>0.1$).

There was also a significant, but less striking difference between the Nanaimo River populations along the second component axis ("approach" and "nip") over all test ages (Fig. 1b, $P=0.05$, df = 1,6); again, interaction levels were highest in First Lake chinook. No differences in agonistic behaviour as summarized by PC2 were found among families nested within populations ($P=0.1$, df = 6,45, Table 3), and all salmon interacted most vigourously at emergence and less so at older ages (age $P=0.006$, Fig 1b). Again, both populations and all families within populations showed no significant differences in the effect of age on agonistic behaviour (all interactions $P>0.1$).

Variation in Salinity Tolerance

At emergence, most salmon survived 24 h exposure to 20 ppt seawater; only three families experienced some mortality: First Lake families 1 and 3 (30% and 37% mortality, respectively) and lower Nanaimo family 1 (17%). At older ages, there were no mortalities in any family in 20 ppt seawater. At emergence and until they were 3 mo old, however, salmon lost weight during 24 h exposure to 20 ppt seawater and the weight loss, both among and within families, was strongly size-dependent with smaller salmon losing proportionately more weight (Fig 2a).

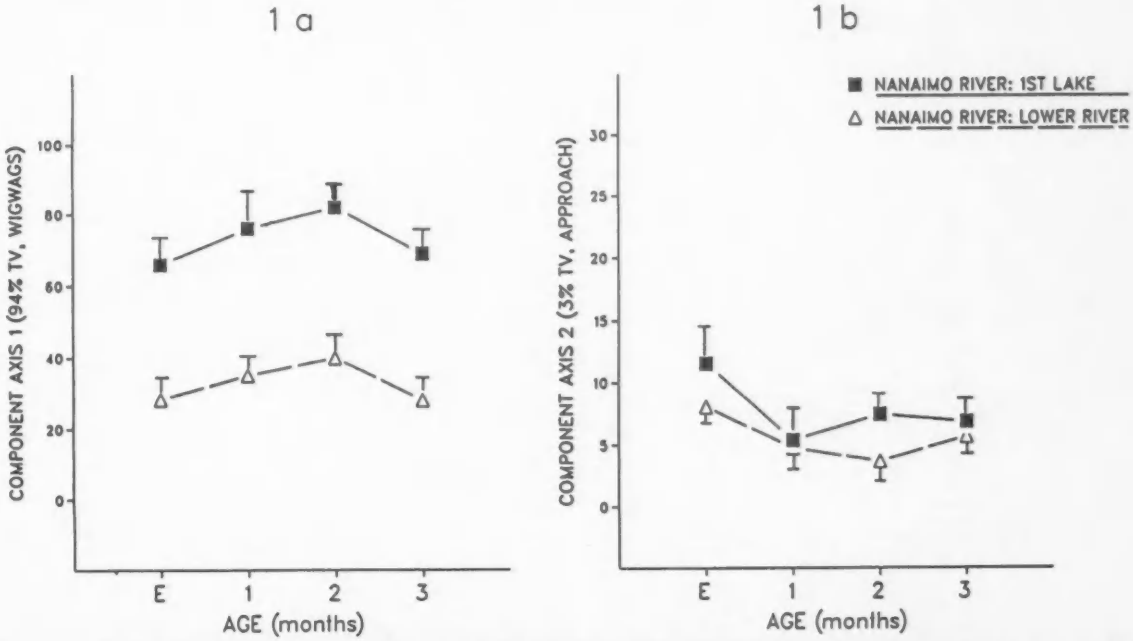

FIG. 1. Mean (± SE) agonistic behaviour scores for juvenile chinook salmon from First Lake and lower Nanaimo River populations over their first 3 mo. 1a = principal component axis 1, 1b = principal component axis 2. For First Lake, $N=7$, 6, 6, and 7 groups of 10 fish at emergence, 1, 2, and 3 mo respectively. Samples sizes at the same ages for lower Nanaimo River chinook are 11, 12, 13, and 15.

TABLE 3. Mean (± SE) agonistic behaviour scores for juvenile chinook from the eight Nanaimo River families averaged over the 3-mo test period.

Family	N	Component PC1	Axis PC2
First Lake			
Family 1	9	73.5 (6.1)	10.3 (1.5)
Family 2	9	66.1 (4.7)	8.3 (0.5)
Family 3	9	78.9 (7.0)	5.4 (1.0)
$\bar{x}$	3	72.8 (3.7)	8.0 (1.4)
Lower Nanaimo			
Family 1	12	30.3 (2.8)	5.6 (0.7)
Family 2	9	27.8 (3.0)	5.9 (0.9)
Family 3	11	42.5 (4.1)	5.4 (1.0)
Family 4	9	26.4 (2.7)	5.8 (0.8)
Family 5	10	36.2 (4.5)	4.9 (0.8)
$\bar{x}$	5	36.6 (3.0)	5.5 (0.2)

Survival after 24 h in 30 ppt seawater was much lower than in 20 ppt and performance was also size-dependent (Fg 2b); again, larger fish performed better. No fish, except for a few from lower Nanaimo family 5, survived 24 h exposure to 30 ppt seawater at emergence, but there was substantial survival in some families at 1 mo, and survival exceeded 75% in lower Nanaimo families 4 and 5 by 2 mo (Fig 3). While linear models adequately explained variation in weight loss ($r^2 = 0.82$) and survival ($r^2 = 0.73$) in terms of size, there is some suggestion that both weight loss and survival increased abruptly at 55–60 mm (SL) or 2.0–3.5 g (Fig 2a,b).

From emergence to 3 mo, salmon from First Lake were 20% smaller than those from the lower Nanaimo and they lost a greater proportion of their initial weights after 24 h in 20 ppt seawater (4.7% vs. 2.6%) although variability among families within populations was greater ($P > 0.05$, df = 1,6 vs. $P < 0.0001$, df = 6,66). When adjusted to a common standard length of 53 mm, salmon from both populations lost a similar amount of weight (3.2% vs. 2.5% respectively, $P = 0.3$, df = 1,6). Significant variation in percentage weight loss in 20 ppt seawater among families within populations, however, persisted after size adjustment ($P = 0.0002$, df = 6,65, Table 4). All chinook lost less weight as they grew older independent of changes in size, but comparisons among age-groups were compromised by heterogeneity of variances and regression slopes (both $P < 0.01$).

Over the 3 mo test period, the larger lower Nanaimo chinook had a higher mean survival rate in 30 ppt seawater (35.7 ± 1.2 vs. 21.2 ± 1.9%), but interfamily differences were again more notable ($P > 0.1$, df = 1,6 vs. $P < 0.0001$, df = 6,66). After adjustment to a common size of 54 mm (SL), however, survival was similar in fish from the two populations, but significant size-independent variation in survival persisted among families nested within populations ($P = 0.0001$, df = 6,65, Table 5). Overall, older (and larger) salmon performed better than younger (and smaller) salmon, but heterogenous error variances among age-groups again compromised size-adjusted comparisons. Lastly, both populations, and all families, increased survival with increasing age in generally similar fashion (all interactions $P > 0.3$, Fig. 3).

Averaged over 3 mo, percentage weight loss in 20 ppt seawater and survival in 30 ppt were highly negatively correlated among the families of salmon ($r = -0.92$, $P < 0.01$, df = 6); families that lost the most weight in 20 ppt seawater also had the lowest survival in 30 ppt. Also, freshwater life history of adult salmon (as inferred from scale analysis) tended to be reflected in the behavioural and physiological performance of their young. While correlations fell short of significance, high

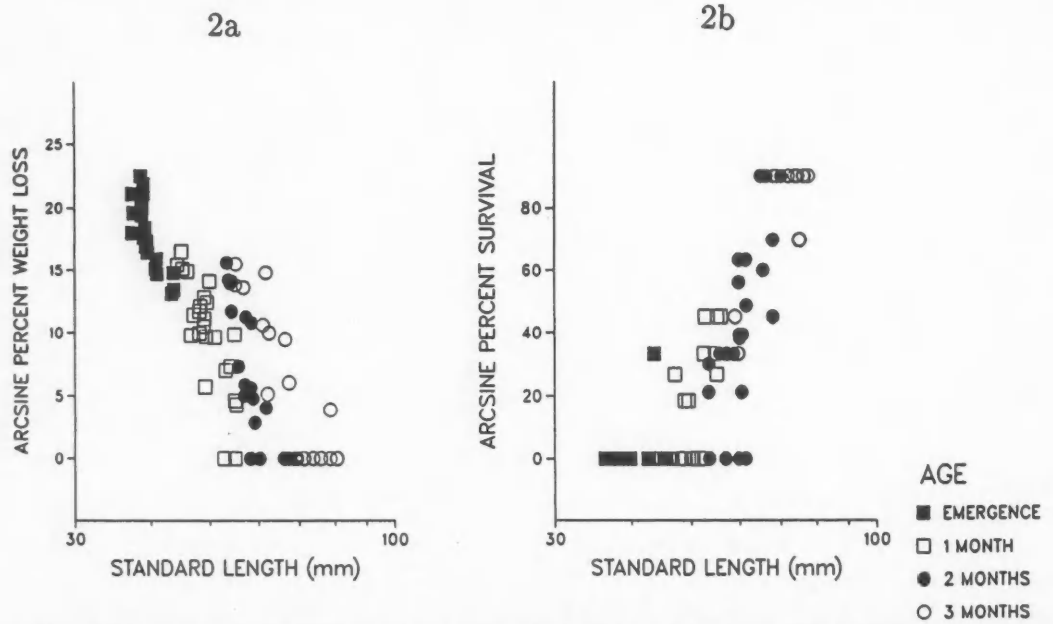

FIG. 2. Relationship between body size (standard length) and measures of salinity tolerance for juvenile chinook salmon from the Nanaimo River over their first 3 mo. 2a = weight loss in 20 ppt seawater vs. standard length, 2b = survival in 30 ppt seawater vs. standard length. Each point is the mean response of 10 fish.

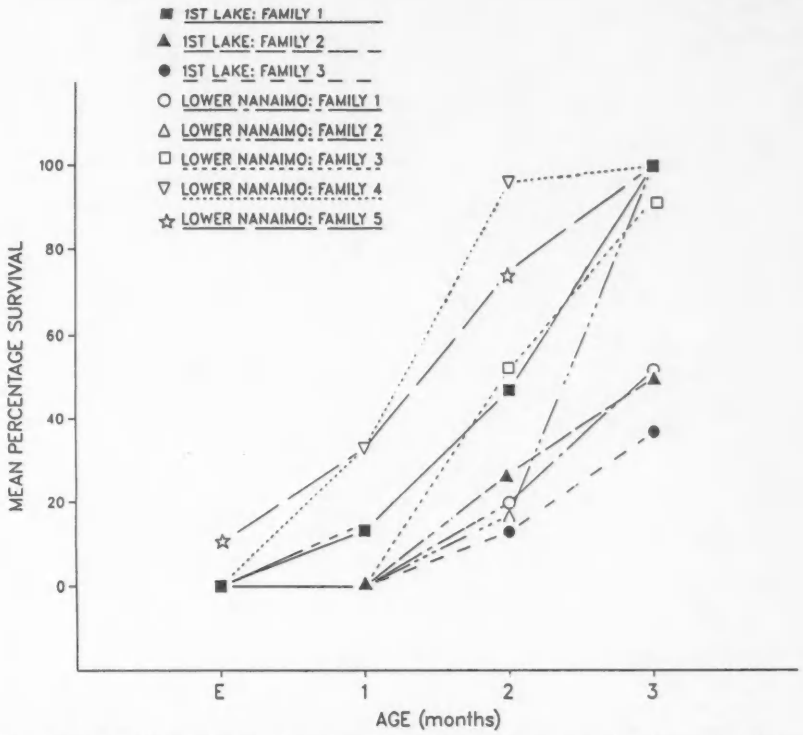

FIG. 3. Changes with age in mean ($N = 3$ groups of 10 fish) percentage survival after 24 h in 30 ppt seawater for eight families of chinook salmon from the Nanaimo River. Closed symbols = First Lake families, open symbols = lower Nanaimo River families.

TABLE 4. Percentage weight loss after 24 h in 20 ppt seawater for juvenile chinook salmon from eight families from the Nanaimo River. For each family, values are raw and size-adjusted (to 53 mm SL) means ($N = 12$, ± SE) over a 3 mo period.

Family	Percentage weight loss	Adjusted percentage weight loss
First Lake		
Family 1	3.14 (1.03)	1.95 (0.72)
Family 2	4.90 (1.51)	3.55 (0.75)
Family 3	6.20 (1.16)	4.10 (0.84)
$\bar{x}$	4.73 (0.89)	3.21 (0.64)
Lower Nanaimo		
Family 1	4.21 (1.06)	3.15 (1.09)
Family 2	2.23 (0.83)	1.31 (0.63)
Family 3	2.88 (1.26)	1.98 (0.63)
Family 4	1.46 (0.70)	2.20 (0.98)
Family 5	2.13 (1.16)	3.85 (1.18)
$\bar{x}$	2.58 (0.47)	2.51 (0.45)

TABLE 5. Percentage survival after 24 h in 30 ppt seawater for juvenile chinook salmon from eight families from the Nanaimo River. For each family, values are raw and size-adjusted (to 54 mm SL) means ($N = 12$, ± SE) over a 3 mo period.

Family	Percentage survival	Adjusted percentage survival
First Lake		
Family 1	38.9 (10.3)	37.0 (3.22)
Family 2	8.71 (6.86)	26.0 (3.46)
Family 3	13.5 (4.90)	22.1 (5.11)
$\bar{x}$	21.33 (8.15)	28.4 (4.52)
Lower Nanaimo		
Family 1	7.4 (4.23)	25.8 (5.14)
Family 2	27.4 (11.4)	30.0 (2.93)
Family 3	33.1 (10.3)	32.6 (2.96)
Family 4	52.0 (11.2)	43.7 (5.91)
Family 5	51.8 (9.6)	43.3 (4.66)
$\bar{x}$	35.7 (10.6)	35.1 (3.64)

freshwater circuli counts of parents (i.e. increased duration of freshwater residence as juveniles) were associated with high levels of agonistic behaviour and high percentage weight loss in 20 ppt seawater, but with low mean survival in 30 ppt seawater of their progeny (all averaged over 3 mo of testing, Table 6).

Discussion

Although behavioural differences among families are not uncommon in salmonids (e.g. Ferguson et al. 1987), my study is one of few to assess behavioural variability among families within populations relative to that between populations. Such designs increase the size of studies on interpopulation variation, but they provide a more rigorous measure of differences between populations. Assessing differences between populations relative to that among families minimizes problems associated with making inferences about populations from data based on observations of young from pooled matings, partic-

TABLE 6. Correlations between parental freshwater life history (scale freshwater circuli counts, FWC), and measures of agonistic behaviour and salinity tolerance of their progeny averaged over 3 mo.

	Female FWC	Male FWC	Mean PC1 score	Mean % weight loss
Male FWC	0.82[a]	—		
Mean PCI score	0.62	0.40	—	
Mean % weight loss	0.60	0.12	0.33	—
Mean % survival	−0.62	−0.64	−0.42	−0.92[a]

[a]$P<0.05$

ularly if the number of parents sampled is small relative to total population size.

Families of juvenile chinook salmon within the two Nanaimo River populations were remarkably similar in their levels of agonistic behaviour from emergence to 3 mo. By contrast, after accounting for among family variation, First Lake salmon had strikingly higher levels of agonistic behaviour than salmon from the lower Nanaimo population (Fig. 1). Since juveniles from the two populations were raised under identical laboratory conditions, my results suggest that this behavioural variability is, at least in part, genetic in origin and is concentrated in diversification between populations of the Nanaimo River.

Carl and Healey (1984) documented morphological and biochemical genetic variation among chinook life history types within the Nanaimo River. The authors concluded, primarily on the basis of the biochemical data, that genetic separation among life history types, and among the populations from which they were presumed to originate, although significant, was weak (Carl and Healey 1984). By contrast, my study revealed differences in agonistic behaviour between Nanaimo populations that were striking. Since these behavioural differences appear to be genetically controlled, my results emphasize the need for caution in interpreting the extent of genetic differentiation between populations from studies based solely on biochemical genetic data which may not reflect genetic variation in quantitative traits (cf. Utter 1981; Barker and Thomas 1987; Lannan et al. 1989). Such considerations can have important management implications. Decisions regarding genetic population "matching" and choice of donor populations for rehabilitation and enhancement programs, that infer genetic similarity from biochemical genetic data, may be unjustified if the same populations differ markedly in behaviour or other quantitative traits. Genetic variation in quantitative traits must be incorporated into such management decisions and Lannan et al. (1989) pointed out the immediate need for practical methods for the measurement of this component of genetic variation.

Differences in agonistic behaviour between First Lake and lower Nanaimo chinook were largely attributable to different durations of "lateral display" behaviour, but less so with respect to overt behavioural acts ("approach," "nip," "charge"). The prominence of display behaviour in elucidating differences between populations in this study is consistent with results for other chinook populations (Taylor 1988, 1990) , for studies of "lake" and "stream" forms of coho salmon (Swain and Holtby 1989) and of different species of charr (Ferguson and Noakes 1982). This consistency suggests that during the divergence of populations, or species, with contrasting life styles (i.e. territorial stream residence vs. a pelagic, roaming life history) the effects of natural selection on agonistic behavioural repertoires are most pronounced for display action patterns.

Salmon from the First Lake population feed in the river for 2–3 mo and then abandon territoriality and emigrate to the estuary (Carl and Healey 1984). By contrast, lower Nanaimo chinook migrate to the estuary as newly emerged salmon (Carl and Healey 1984). Territoriality is an important component of extended stream residence in salmonids because it enhances survival by increasing feeding success and providing protection from predation (Symons 1974; Keenlyside 1979; Fausch 1984; Grant and Noakes 1988). In comparisons between closely related species, or between populations within species, those with life histories characterized by extended territorial stream residence also show higher degrees of agonistic behaviour than those species or populations characterized by early migration to estuarine, marine, or lacustrine habitats (Hoar 1951; Ferguson and Noakes 1982; Taylor and Larkin 1986; Bakker and Feuth-de Bruijn 1988; Swain and Holtby 1989). Given an association between freshwater life history and inherited differences in agonistic behaviour between First Lake and lower Nanaimo salmon, my results strengthen the idea that selection, acting through the functional relationships among duration of stream residence, territoriality, and agonistic behaviour, is responsible for differences in agonistic behaviour between populations with contrasting life styles.

Covariation between size and salinity tolerance and differences in these traits between populations may also be related to the contrasting life histories of First Lake and lower Nanaimo salmon. Female salmon from the lower Nanaimo tend to be larger than those from First Lake and thus, would tend to produce larger young at emergence because of positive relationships among female size, egg size, and size at emergence in salmon (Table 1; Fowler 1972; Healey and Jordan 1982; Beacham and Murray 1985). Given the importance of size to performance in seawater (Fig. 2; Conte et al. 1966; Wagner et al. 1969; McCormick and Naiman 1984; Heifetz et al. 1989), the relatively large size of lower Nanaimo fish should enhance their survival in the estuary where salinities commonly exceed 20 ppt (Healey 1980). By contrast, First Lake chinook typically do not enter the estuary until they have fed in the river for 2–3 mo and reached a size of 2–3 g, when salinity tolerance is much enhanced over that of newly-emerged salmon (Healey and Jordan 1982; Carl and Healey 1984; Fig. 2). Therefore, if there is a genetic component to female size at maturity or egg size (Aulstad and Gjedrem 1973; Beacham and Murray 1988), then it is plausible that the large size of lower Nanaimo salmon represents an adaptation to their estuarine rearing environment.

Notwithstanding this possibility, differences in both measures of salinity tolerance between populations were subordinate to differences among families within populations (Table 4, 5). It is probable, however, that a larger sample of families, particularly from the First Lake population, would have detected significant differences in seawater adaptability at the population level. For instance, pooled progeny of crosses made with 15 female and 10 male salmon from the Squamish River, a stream-type population from southwestern B.C., lost significantly more weight and had significantly lower survival in seawater than salmon from both Nanaimo River populations (Taylor 1989).

In addition to the results of my study, Beacham and Murray (1989) detected significant interfamily variation in size at emergence in three other British Columbia populations of chinook salmon. Since size was an important determinant of weight loss and survival in seawater (Fig. 2), size-dependent interfamily variation in salinity tolerance is probably common in chinook, at least for newly-emerged salmon. Furthermore, although dif-

ferences in salinity tolerance were greatest before adjustment to a common size, differences persisted after adjustment by covariance analysis which suggests that these families, raised under identical conditions, may differ in a size-independent genetic factor that influences salinity tolerance. Consequently, my results illustrate the importance of considering maternal effects and interfamily differences in the design of studies intended to investigate physiological variability among populations (cf. Beachman and Murray 1986a, 1986b).

In conclusion, chinook salmon from different populations in the Nanaimo River show inherited differences in juvenile phenotype that probably result from selection associated with distinct life styles. Swain and Holtby (1989) documented a similar tendency for juvenile coho salmon and noted that adaptive differentiation in coho may exist on a surprisingly small geographic scale. Behavioural and physiological diversification of Nanaimo River chinook salmon provide further evidence of microgeographic divergence in Pacific salmon and illustrate that populations may be strikingly different in quantitative traits in spite of apparently low levels of biochemical genetic divergence.

Acknowledgments

I thank P. Preston and the staff of the Nanaimo River Community Salmon Hatchery for assistance during the spawning and incubation phases of this study and P. A. Larkin and M. C. Healey for helpful discussions. I appreciate the assistance of two reviewers in improving this paper. Research funding was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) operating grant awarded to P. A. Larkin, and I was supported by an NSERC postdoctoral fellowship while writing this paper.

References

Allendorf, F. W., and N. Ryman. 1987. Genetic management of hatchery populations, p. 121–140. *In* N. Ryman and F. M. Utter [ed.] Population Genetics and Fishery Management. University of Washington Press, Seattle. 420 p.

Aulstad, D., and T. Gjedrem. 1973. The egg size of the salmon (*Salmo salar*) in Norwegian rivers. Aquaculture 2: 337–341.

Bakker, T., and E. Feuth-de Bruijn. 1988. Juvenile territoriality in sticklebacks (*Gasterosteus aculeatus* L.) Anim. Behav. 36: 1556–1558.

Bams, R. A. 1976. Survival and propensity for homing as affected by presence or absence of locally adapted paternal genes in two transplanted pink salmon populations. J. Fish. Res. Board Can. 31: 1379–1385.

Barker, J. S. F., and R. H. Thomas. 1987. A quantitative genetic perspective on adaptive evolution, p. 3–23. *In* V. Loeschcke [ed.] Genetic Constraints on Adaptive Evolution. Springer–Verlag, Berlin.

Beacham, T. D. 1988. A genetic analysis of early development in pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) at three different temperatures. Genome 30: 89–96.

Beacham, T. D., and C. B. Murray. 1989. Variation in developmental biology of sockeye salmon (*Oncorhynchus nerka*) and chinook salmon (*O. tshawytscha*) in British Columbia. Can. J. Zool. 67: 2081–2089.

1988. A genetic analysis of body size in pink salmon (*Oncorhynchus gorbuscha*). Genome 30: 31–35.

1986a. Comparative developmental biology of pink salmon, *Oncorhynchus gorbuscha*, in southern British Columbia. J. Fish Biol. 28: 233–246.

1986b. Comparative developmental biology of chum salmon, *Oncorhynchus keta*, in the Fraser River, British Columbia. Can. J. Fish. Aquat. Sci. 43: 252–262.

1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon, (*Oncorhynchus keta*), from the Nitinat River, British Columbia. Can. J. Fish. Aquat. Sci. 42: 1755–1765.

Blackburn, J., and W. C. Clarke. 1987. Revised procedure for the 24 hr seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1515: 35 p.

Carl, L. M., and M. C. Healey. 1984. Differences in enzyme frequency and body morphology among three juvenile life history types of chinook salmon (*Oncorhynchus tshawytscha*) in the Nanaimo River, British Columbia. Can. J. Fish. Aquat. Sci. 41: 1070–1077.

Clutter, R. I., ad L. E. Whitesel. 1956. Collection and interpretation of sockeye salmon scales. Int. Pac. Salmon Fish. Comm. Bull. 9: 159 p.

Conte, F. P., H. H. Wagner, J. Fessler, and C. Gnose. 1966. Development of osmotic and ionic regulation in juvenile coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. 18: 1–15.

Fausch, K. D. 1984. Profitable stream positions for juvenile salmonids: relating specific growth rate to net energy gain. Can. J. Zool. 62: 441–451.

Ferguson, M. M., R. G. Danzmann, and F. W. Allendorf. 1987. Adaptive significance of developmental rate in rainbow trout: an experimental test. Biol. J. Linn. Soc. 33: 205–216.

Ferguson, M. M., and D. L. G. Noakes. 1982. Genetics of social behaviour in charrs (*Salvelinus spp.*). Anim. Behav. 30: 128–130.

Fowler, L. G. 1972. Growth and mortality of fingerling chinook as affected by egg size. Prog. Fish.-Cult. 34: 66–69.

Fox, D. J., and K. E. Guire. 1976. Documentation for MIDAS. 3rd. ed. Statistical research laboratory, University of Michigan, Ann Arbour, MI.

Grant, J. W. A., and D. L. G. Noakes. 1988. Aggressiveness and foraging mode of young-of-the-year brook charr, *Salvelinus fontinalis* (Pisces: Salmonidae). Behav. Ecol. Sociobiol. 22: 435–445.

Healey, M. C. 1980. Utilization of the Nanaimo River estuary by juvenile chinook salmon, *Oncorhynchus tshawytscha*. U.S. Fish and Widl. Ser. Fish. Bull. 77: 653–668.

Healey, M. C., and W. R. Heard. 1984. Inter- and intrapopulation variation in the fecundity of chinook salmon (*Oncorhynchus tshawytscha*) and its relevance to life history theory. Can. J. Fish. Aquat. Sci. 41: 476–483.

Healey, M. C., and F. P. Jordan. 1982. Observations on juvenile chum and chinook and spawning chinook in the Nanaimo River, British Columbia during 1975–81. Can. Manuscr. Rep. Fish. Aquat. Sci. 1659: 31 p.

Heifetz, J., S. W. Johnson, K. V. Koski, and M. L. Murphy. 1989. Migration timing, size, and salinity tolerance of sea-type sockeye salmon (*Oncorhynchus nerka*) in an Alaskan estuary. Can. J. Fish. Aquat. Sci. 46: 633–637.

Hoar, W. S. 1951. The behaviour of chum, pink and coho salmon in relation to their seaward migration. J. Fish. Res. Board Can. 8: 241–263.

Huntingford, F. A. 1976. An investigation of the territorial behaviour of the three-spine stickleback (*Gasterosteus aculeatus*) using principal components analysis. Anim. Behav. 24: 822–834.

Keenleyside, M. A. H. 1979. Diversity and adaptation in fish behaviour. Zoophysiology Springer-Verlag, Berlin. 208 p.

Lannan, J. E., G. A. E. Gall, J. E. Thorpe, C. E. Nash, and B. E. Ballachey. 1989. Genetic resource management of fish. Genome 31: 798–804.

McCormick, S. D., and R. J. Naiman. 1984. Osmoregulation in the brook trout, *Salvelinus fontinalis*-II. Effects of size, age and photoperiod on seawater survival and ionic regulation. Comp. Biochem. Physiol. 79A: 17–28.

Nishikawa, K. C. 1985. Competition and the evolution of aggressive behavior in two species of terrestrial salamanders. Evolution 39: 1282–1294.

Parry, G. 1960. The development of salinity tolerance in the salmon, *Salmo salar* (L.), and some related species. J. Exp. Biol. 37: 425–434.

Quinn, T. P. 1985. Homing and the evolution of sockeye salmon (*Oncorhynchus nerka*). Contrib. Mar. Sci. 27 (Suppl.): 353–366.

Raleigh, R. F. 1971. Inate control of migrations of salmon and trout fry from natal gravels to rearing areas. Ecology 52: 291–297.

Ricker, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations, p. 19–160. *In* R. C. Simon and P. A. Larkin [ed.] The stock concept in Pacific salmon. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C. 213 p.

Riddell, B. E., and W. C. Leggett. 1981. Evidence of an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 38: 308–320.

Rosenau, M. L., and J. D. McPhail. 1987. Inherited difference in agonistic behavior between two populations of coho salmon. Trans. Am. Fish. Soc. 116: 646–655.

Schluchter, M. D., and J. A. Lichatowich. 1977. Juvenile life histories of Rogue River spring chinook salmon (*Oncorhynchus tshawytscha*) (Walbaum), as determined by scale analysis. Oregon Dept. Fish and Wildl. Info. Rep. Ser. Fish. 77–5: 24 p.

Swain, D. P., and B. Holtby. 1989. Differences in morphology and agonistic behavior in coho salmon (*Oncorhynchus kisutch*) rearing in a lake or its tributary stream. Can. J. Fish. Aquat. Sci. 46: 1406–1414.

SYMONS, P. E. K. 1974. Territorial behavior of juvenile Atlantic salmon reduces predation by brook trout. Can. J. Zool. 52: 677–679.

TAYLOR, E. B. 1990. Phenotypic correlates of life history variation in juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). J. Animal Ecol. 59: 455–468.

TAYLOR, E. B. 1989. Adaptive diversification of juvenile life histories in the chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). Ph.D. thesis, University of British Columbia, Vancouver, B.C. 283 p.

1988. Adaptive variation in rheotactic and agonistic behavior in newly-emerged fry of chinook salmon, *Oncorhynchus tshawytscha*, from ocean- and stream-type populations. Can. J. Fish. Aquat. Sci. 45: 237–243.

TAYLOR, E. B., AND P. A. LARKIN. 1986. Current response and agonistic behavior in newly-emerged fry of chinook salmon, *Oncorhynchus tshawytscha*, from ocean- and stream-type populations. Can. J. Fish. Aquat. Sci. 43: 565–573.

TAYLOR, E. B., AND J. D. MCPHAIL. 1985. Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42: 2029–2033.

TITUS, R. G., AND H. MOSEGAARD. 1989. Smolting at age 1 and its adaptive significance for migratory trout, *Salmo trutta* L., in a small Baltic-coast stream. J. Fish. Biol. 35: (Suppl. A): 351–354.

UTTER, F. M. 1981. Biological criteria for definition of species and distinct intraspecific populations of anadromous salmonids under the U.S. Endangered Species Act of 1973. Can. J. Fish. Aquat. Sci. 38: 1626–1635.

WAGNER, H. H., F. P. CONTE, AND J. L. FESSLER. 1969. Development of osmotic and ionic regulation in two races of chinook salmon, *Oncorhynchus tshawytscha*. Comp. Biochem. Physiol. 29: 325–341.

WITHLER, R. E., W. C. CLARKE, B. E. RIDDELL, AND H. KREIBERG. 1987. Genetic variation in freshwater survival and growth of chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 64: 85–96.

ZAR, J. H. 1984. Biostatistical Analysis. 2nd ed. Prentice–Hall Inc., Eaglewood Cliffs, NJ. 718 p.

Importance of Smolt Size and Early Ocean Growth to Interannual Variability in Marine Survival of Coho Salmon (*Oncorhynchus kisutch*)[1]

L. Blair Holtby, Bruce C. Andersen, and Ronald K. Kadowaki

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6 Canada

Holtby, L. B., B. C. Andersen, and R. K. Kadowaki. 1990. Importance of smolt size and early ocean growth to interannual variability in marine survival of coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 47:2181–2194.

The importance of smolt size and early ocean growth to the marine survival of coho salmon was examined over a 17-yr period at Carnation Creek, British Columbia. Comparisons of overall marine survival were made both between-years, using two smolt age-groups of different mean sizes, and within-years using observed smolt size distributions and smolt size distributions back-calculated from the scales of returning adults. Large size did not confer a consistent survival advantage but large smolts did survive better in years when marine survival was relatively poor. Marine survivals were strongly correlated with early ocean growth as estimated by the spacing of the first five ocean circuli on the scales of returning adults. Marine survival and early ocean growth were positively correlated with ocean conditions indicative of strong upwelling along the northwest coast of Vancouver Island. Neither smolt survival nor early ocean growth were correlated with regional coho smolt production. Our observations suggest that interannual variability in smolt survival was being driven by ocean conditions that determined smolt growth rates which subsequently affected the susceptibility of smolts to a size-selective predator.

L'importance que revêtent pour la survie en mer du saumon coho la taille des saumoneaux de descente et leur croissance durant le début de leur séjour océanique a été étudiée sur une période de 17 ans à Carnation Creek, en Colombie-Britannique. Les comparaisons des taux de survie d'ensemble en milieu marin ont été effectuées à la fois entre les années en prenant deux groupes d'âge de saumoneaux de taille moyenne différente et à l'intérieur de chaque année sur la base des distributions de taille observées chez les saumoneaux de descente et calculées rétroactivement grâce aux écailles des adultes revenus. On a pu déterminer qu'une grande taille ne confère pas un avantage systématique du point de vue de la survie mais les grands saumoneaux ont néanmoins mieux survécu les années où le taux de survie en mer a été relativement faible. On a aussi pu constater une forte corrélation entre les taux de survie en mer et la phase initiale de croissance océanique telle qu'estimée en fonction de l'espacement des cinq premiers cercles océaniques sur les écailles des adultes revenus. Une corrélation positive a également été établie entre d'une part la survie en mer et les premiers stades de croissance dans l'océan et, d'autre part, des conditions océaniques révélant une forte résurgence d'eau froide de long de la côte nord-ouest de l'île de Vancouver. Ni la survie des saumoneaux de descente ni les premiers stades de la croissance océanique n'ont été mis en corrélation avec la production régionale de saumoneaux de descente coho. Nos observations donnent à penser que la variabilité inter-annuelle que l'on relève dans les taux de survie des saumoneaux de descente est surtout fonction de conditions océaniques ayant déterminé les taux de croissance des saumoneaux, ce qui s'est ensuite répercuté sur le degré auquel les saumoneaux ont été attaqués par un prédateur choisissant ses proies en fonction de leur taille.

Received December 4, 1989
Accepted May 24, 1990
(JA387)

Reçu le 4 décembre 1989
Accepté le 24 mai 1990

Considerable mortality of Pacific salmon (*Oncorhynchus* sp) smolts occurs during a brief period after their entry into the ocean (Parker 1971; Mathews and Buckley 1976; Walters et al. 1978; Healey 1982). Several hypotheses have been proposed to explain variations in smolt survival. The "ocean–productivity" hypothesis links periodically unfavorable ocean conditions with slow fish growth and poor fish condition, and thus greater vulnerability to predation (Fisher and Pearcy 1988). Correlations between smolt survival and ocean upwelling in the first year of ocean residence are cited in support of this hypothesis (Scarnecchia 1981; Nickelson 1986). The "density–dependence" hypothesis relates the entry of large numbers of smolts into the ocean with food limitation, slowed growth and increased predation or starvation (Foerster 1954; Peterman 1978; Walters et al. 1978). The "predation–intensity" hypothesis relates variations in smolt survival solely to levels of predation. Predation levels can vary because of a functional response by the predator to changes in the abundance of alternative prey species (Fisher and Pearcy 1988), because of changes in the distributions of predators or their prey (Ware and McFarlane 1988), or because of absolute changes in the abundance of predators.

Both the "ocean-productivity" hypothesis and the "density-dependence" hypothesis assume that a significant component of marine mortality of smolts is size-dependent. Consequently, a corollary of both hypotheses is that smolt mortality is inversely

[1]Contribution No. 151 of the Carnation Creek Experimental Watershed Project.

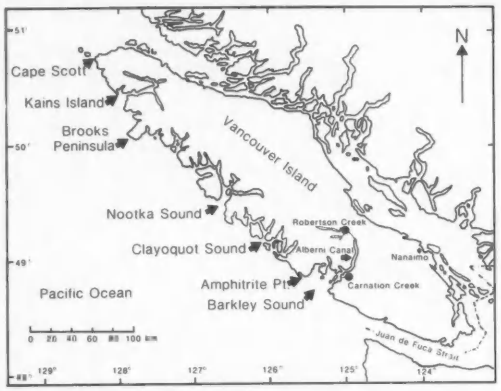

FIG. 1. The west coast of Vancouver Island.

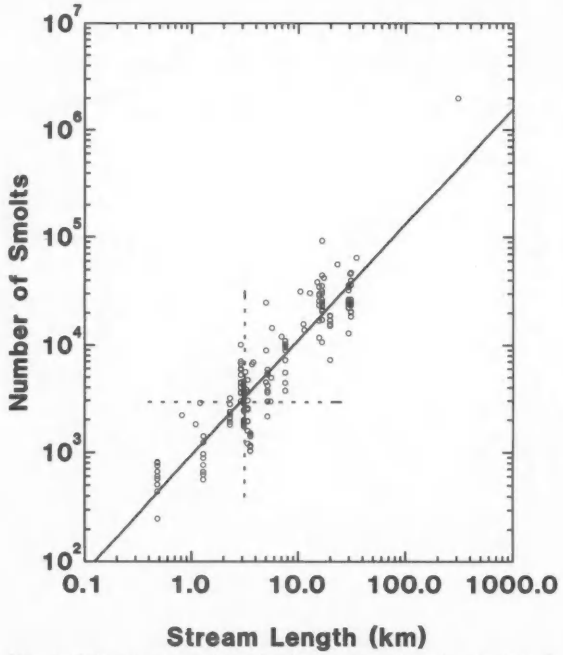

FIG. 2. Coho salmon smolt production as a function of stream length for 36 streams on the west coast of North America. For most streams several years of data were available. The intersection of the dashed lines indicates the average smolt production at Carnation Creek before 1976.

related to growth rate. In contrast, the "predation–intensity" hypothesis as presented by Fisher and Pearcy (1988) explicitly states that marine survival of smolts is independent of smolt size and growth and thus that smolt survival is independent of smolt densities and the direct effects of variable ocean productivity.

In this paper we describe relationships between smolt survival, smolt size, and early marine growth for a wild coho salmon (*Oncorhynchus kisutch*) population over the period 1971–1987 (smolt years). This examination will discriminate between the "predation–intensity" hypothesis on one hand and the "ocean–productivity" and "density–dependence hypotheses" on the other. To discriminate between the latter two hypotheses, we will test for density–dependent effects on survival and growth and describe relationships between smolt survival, early ocean growth, and oceanographic variables thought to be indicative of ocean conditions in the ocean rearing area.

Methods

Study Site

Carnation Creek is a small stream on the west coast of Vancouver Island that drains into Barkley Sound (Fig. 1). The stream supports a run of several hundred coho salmon and produces from 2500–4500 smolts·yr^{-1}. On average 75% (range 43–97%) of the smolts produced spend 1 yr in the stream (1^+ smolts), the remainder spend 2 yr (2^+ smolts). Beginning in 1970, Carnation Creek was the site of a long-term study of the effects of clear-cut logging on salmon production. Extensive descriptions of the study are available (Chamberlain 1987). Most of the fisheries data used here have been summarized by Andersen (1983, 1984, 1985, 1987; B.C. Andersen, unpubl. data). The effects of logging on the Pacific salmon populations of the stream have been summarized by Holtby (1988) and Holtby and Scrivener (1989).

Fish Enumeration and Aging

Adult coho salmon were captured at a fence located 75 m upstream of the mean high tide level from late September through early March from 1972 through 1988. In the fall of 1971, only a portion of the fish were captured at a fence and total escapement was estimated by mark-recapture. All fish, including jacks (males that return after 6 mo in the ocean compared to the usual 18 mo), were measured to the nearest cm, sexed, and passed upstream of the fence. Between eight and 30 scales were taken from the preferred area (left side, just behind the dorsal fin and just above the lateral line; Clutter and Whitesel (1956). Because of the proximity of the counting fence to the ocean and immature condition of most adults, scale resorption was minimal. Two or three selected scales were mounted on gummed cards. Plastic impressions of the scales were made using standard procedures (Clutter and Whitesel 1956). Freshwater ages (either 1 or 2 yr, denoted by 1^+ or 2^+, respectively) and marine ages (either zero or one, respectively indicating 6 or 18 mo spent in the ocean) were determined by examining those impressions under $75\times - 150\times$ magnification. Fish that could not be aged, either because the freshwater zone of all scales taken were regenerated, or because the fish escaped during sampling, were apportioned between the two freshwater age-classes, according to the measured age composition by sex and marine age. In all years but 1971, at least 90% of the fish were aged.

Fish from Carnation Creek are caught in their second summer of ocean life in an intensive troll fishery off the west coast of Vancouver Island and the Queen Charlotte Islands (Argue et al. 1987). Jacks are not subject to the fishery and it is possible that more 2^+ than 1^+ smolts became jacks because of their larger size (Bilton et al. 1982). Consequently, an age composition at return calculated by summing jack and large adult returns may have been biased toward the older age-group. Since exploitation rates of Carnation Creek coho salmon were not measured, we assumed that they were the same as exploitation

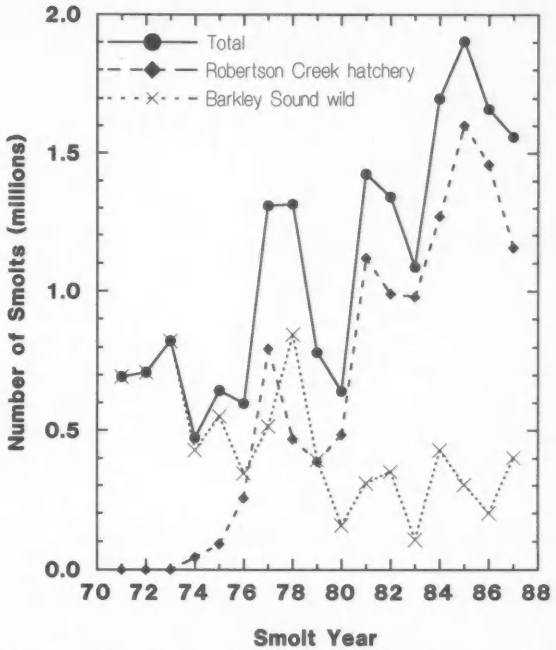

FIG. 3. Components of the estimated total coho salmon smolt production in Barkley Sound 1971–87.

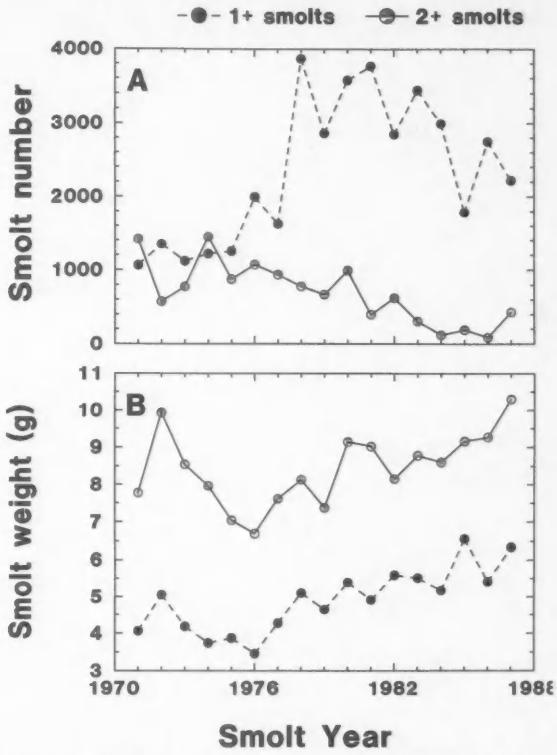

FIG. 4. (A) Numbers of smolts and (B) smolt weights by freshwater age group vs. smolt year.

rates measured for coho salmon at the nearby Robertson Creek hatchery from (return year) 1975 to 1988. Exploitation rates were obtained from the Department of Fisheries and Ocean's mark-release database. Exploitation rates for the three return years prior to 1975 were set equal to the overall average exploitation rate of 65%. Female and non-jack male returns were expanded by the inverse of one minus the estimated exploitation rate before summing with the jack returns from the previous year. The age composition of all returning fish (adult females, jack, and adult males) was then calculated using the expanded numbers.

Outgoing smolts were captured in the spring at the same fence. In most years the forklengths (FL) of more than 95% of smolts were measured to the nearest mm. In 1971, 71 of the smolts were measured. At 7–10 d intervals sub-samples of smolts were weighed to the nearest 0.05 g and scales were taken from the preferred area, mounted between glass slides, and later examined under magnification to determine freshwater age. The age composition of the run was calculated by applying the proportion in the two age-classes to the total run in size-classes of approximately 5 mm. In many years, and particularly in later ones, scales from smaller fish (<70 mm) were under-sampled because their age was known with near certainty. At least 2%, and in most years 10%, of the smolts were aged.

Marine survivals of smolts by freshwater age-group and smolt year were calculated by dividing the expanded return by the smolt number. The arithmetic difference between the marine survivals of the 2^+ and 1^+ smolts of the same smolt year was termed the "2^+ survival differential," and was used to examine under what conditions survivals of the two age-groups differed. The age compositions of the smolts and adults were expressed as the proportions comprised of the younger freshwater age group (age 1^+).

Back-Calculation of Smolt Size from Adult Scales

A demarcation between the freshwater and marine zones can usually be readily seen on scales of adult coho salmon. Circuli in the marine zone are further apart and more robust than those in the freshwater zone, and the two regions are usually separated by a "saltwater entry" check consisting of several fine and closely spaced or intertwined circuli. We used many simultaneous examinations of smolt scales and adult scales from the same cohort to learn how to properly identify the demarcation region. We then examined smolt scales magnified 75×, determined freshwater age, and measured the scale radius (the origin to the scale edge) to within 0.01 mm along an axis 30° to the anterior-posterior axis. At least two scales were measured per fish and the scale measurements were recored along with the age and FL (in mm). A total of 1210 scales was examined (44 to 146 per year for 14 yr). Forklength was regressed on scale radius by year and by freshwater age-class (1^+ or 2^+ yr old). A similar procedure was followed with the adult scales: two scales were examined per fish, the radius was measured along the same 30° axis, and the smolt FL was calculated from the average radius. All adult scales for which a freshwater age could be determined were processed. Adult scale radii were corrected upward by 2.0% to adjust for shrinkage occurring during scale preparation (Rankis 1987). Frequency distributions of smolt sizes based on back-calculation were corrected for the troll fishery harvest as described for the adult age composition. It uncorrected for the fishery harvest, then the back-calculated distribution of smolt sizes could have been biased toward larger

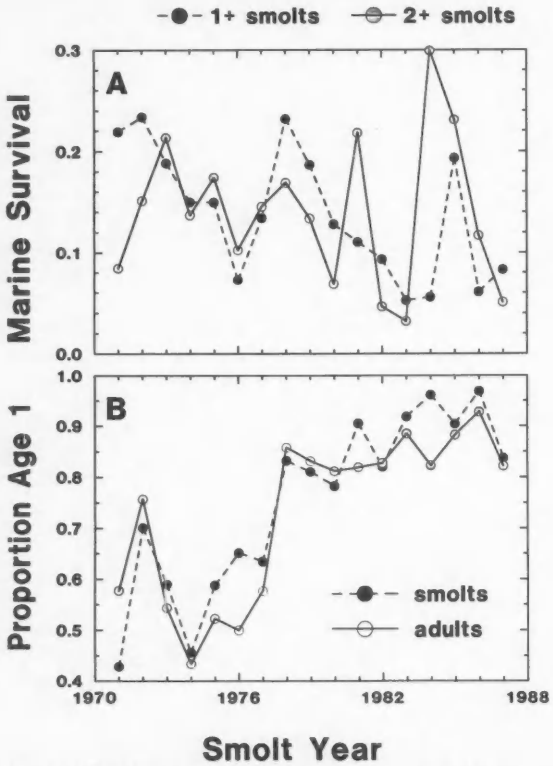

FIG. 5. (A) Marine survival by freshwater age group and (B) proportion of freshwater age 1^+ fish in smolts and adults vs. smolt year.

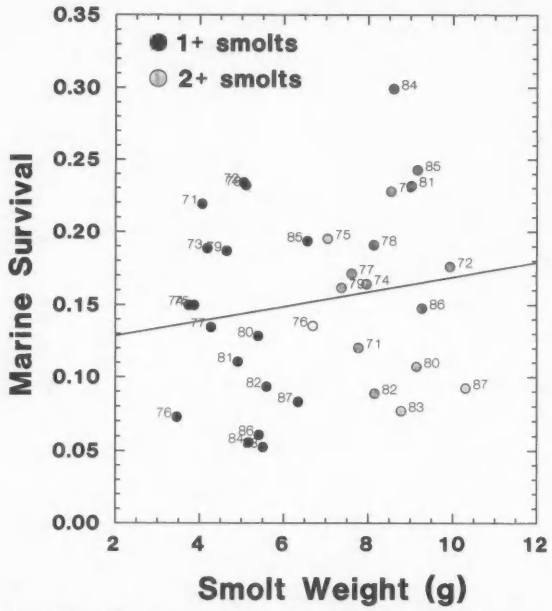

FIG. 6. Marine survival versus smolt weight for both freshwater age groups with fitted regression line.

smolts. Mean smolt sizes derived from the observed and back-calculated size distributions were compared with *t*-tests. Adult scales from smolt years 1974, 1977, and 1980 were not processed.

Determination of Effects of Smolt Size on Smolt Survival

The effect of smolt size on survival was first examined by comparing, within smolt years, the marine survivals of the age 1^+ and age 2^+ smolts. We used paired *t*-tests to compare the marine survivals of age 1^+ and age 2^+ smolts and the proportion of age 1^+ fish in the smolts and returning adults. We regressed survivals of both age-groups on smolt weight to test for an overall effect of size on survival. Although these tests assumed that the only difference between the two age-groups of smolts was size (i.e. there were no behavioural or physiological differences due to age), they required only that fish were correctly aged. The second approach consisted of back-calculating smolt size from measurements taken from adult scales and comparing, within smolt years, the observed size distribution with the expected distribution based on the smolt size-frequency distribution. The effects of smolt size on survival within-years were estimated by regressing smolt survivals on smolt FL within 5 mm length-classes. Length-classes were excluded when observed returns were less than five fish or where the smolt survival was undefined (observed return >0 when expected return = 0). The slope of the regression line ($\beta_{survival}$) indexed the effect of smolt length on survival. A positive slope indicated that survival increased with increasing smolt length and the steeper the slope, the greater was the survival advantage of larger size.

Marine Growth Rates

Estimates of early marine growth were made by measuring the average inter-circular distance over the first five ocean circuli. Inter-circular distances were measured at the same time other scale measurements were taken. Inter-circular distances averaged over the first 2–10 circuli were very similar. Inter-circular distance is correlated with growth rate over an approximately 60-d period after ocean entry (Fisher and Pearcy 1988).

Juvenile Coho Smolt Production in Barkley Sound

No direct estimates of coho smolt production for the Barkley Sound region were available. Average wild production of coho salmon from the region was estimated by first predicting average smolt production for each of the 62 streams in the region (Brown et al. 1987) with the relationship:

$$(1)\quad N_{smolts} = 941.4 \times \text{stream length (km)}^{1.074}\ (n = 168,\ r^2_{adjusted} = 0.84;\ \text{Fig. 2}).$$

The relationship was determined from data presented by Armstrong and Argue (1977), Argue et al. (1979), Holtby and Hartman (1982), and Baranski (1989). Inter-annual variability in that average was estimated by using the process-model of smolt production developed for the Carnation Creek population (Holtby 1988; Holtby and Scrivener 1989). The model predicted smolt production for Carnation Creek in the absence of logging for each smolt year between 1971 and 1987. Predicted smolt productions were divided by the overall mean predicted smolt production from Carnation Creek to give annual scaling factors. The estimated average smolt production from the region was then scaled to give an estimated wild smolt production by

smolt year (Fig. 3). Beginning in 1974, a hatchery on Robertson Creek released up to 1.6 million coho smolts into the Alberni Canal at the head of the Sound. Hatchery production and estimated wild production were summed to give an estimated total smolt production (Fig. 3).

The reliability of our estimation procedure is uncertain, but we think that it should have revealed significant trends in wild smolt production for the following reasons. First, the relationship between stream length and average smolt production (Eq. 1) is strong and predicts average smolt production from Carnation Creek accurately. Carnation Creek is typical in size of streams in the Barkley Sound area. Second, the process models (Holtby 1988; Holtby and Scrivener 1989) have accurately predicted overall trends in coho catch off Vancouver Island over the period 1960–79 (Holtby and Scrivener 1989). Holtby and Scrivener (1989) concluded that the process model had captured the important components of interannual variability. Third, the abundances of juvenile coho salmon in four streams in the Barkley Sound region, including Carnation Creek, covaried over a 12-yr period (Holtby et al. 1984), suggesting that factors determining coho abundance in those Barkley Sound streams were operating on a regional scale.

Ocean Environment Data

Thomson et al. (1989) have characterized the physical oceanography off the west coast of Vancouver Island with four temporal domains. During the winter domain, from October through February, prevailing winds from the southeast and efflux of freshwater from the coastal inlets combine to produce a broad coastal current flowing poleward. Sometime during February through May, the spring transition domain, prevailing winds switch to the northwest and freshwater outflow from the inlets is reduced. Shelf-break currents along the continental margin are established flowing counter to the Vancouver Island Coastal Current. The coastal current continues to flow poleward over the continental shelf, driven by Fraser River outflows through the Juan de Fuca Strait (Thomson et al. 1989). Simultaneously, conditions favourable to upwelling are established along the continental margin. Early and abrupt transitions weaken the coastal current and may lead to increased transport of upwelled water onto the continental shelf north of Barkley Sound. Late or sporadic transitions prolong the flow of southerly surface waters poleward in the coastal current (Thomson et al. 1989). From May through September, the coastal current is driven by outflow from the Juan de Fuca Strait. To the north of Barkley Sound, the coastal current prevents onshore transport of water upwelling along the shelf-break. The summer domain is also characterized by strong upwelling off the northwest tip of Vancouver Island and transport of the upwelled water transported southward by a shelf-break current flowing between Cape Scott and the Brooks Peninsula. Interactions between this current and the poleward flowing coastal current are poorly understood (Thomson et al. 1989). During the fall transition domain from September through October, conditions of the winter domain are reestablished.

Peak smolt emigration from Carnation Creek occurs in late April and early May (Holtby et al. 1989), simultaneously, or as much as 12 wk after the spring transition. Coho smolts originating in Barkley Soud are thought to migrate northward along the coast of Vancouver Island reaching the northwest tip of the island within 3 mo of leaving Barkley Sound (K. Groot, Pacific Biological Station, Nanaimo, B.C., pers. comm.). Based on the oceanography of the coastal waters and the migration timing and pathways of Barkley Sound coho salmon, we examined correlations between marine survivals and growth rates and four time series of sea-surface salinity and temperature data from the Amphitrite Point and Kains Island lighthouses (Fig. 1; Giovando 1985; D. Blackbourn, Pacific Biological Station, Nanaimo, B.C., pers. comm.) and two time series of the coastal upwelling indices at points off the west coast of Vancouver Island. The timing and abruptness of the spring transition were indexed with salinities and temperatures at Amphitrite Point during May and by the magnitude of the coastal upwelling index at 48°N, 125°W during May (Bakun 1973; Mason and Bakun 1986; D. Mallicoate, Pacific Fisheries Environmental Group, N.M.F.S., P.O. Box 831, Monterey, CA 93942, pers. comm.). Relatively high salinities, low temperatures and large upwelling indices would indicate an early spring transition, a weak coastal current during the transition, and cross-shelf transport of water upwelled along the shelf margin. We hypothesized that those conditions should have resulted in favourable growth conditions for salmon smolts entering Barkley Sound and migrating north during May. Ocean conditions during the coho salmon's first summer were indexed by sea-surface salinities and temperatures at Kains Island (Fig. 1) and by the magnitude of

TABLE 1. Comparisons of observed mean smolt forklengths (FL in mm) with expected mean FL back-calculated from adult scales. H_0: no difference in mean sizes; $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

	Age 1⁺ FL (mm)			Age 2⁺ FL (mm)			Overall FL (mm)		
Smolt year	Observed		Expected	Observed		Expected	Observed		Expected
1971	74.3	>***	70.7	96.7	=	96.3	83.8	=	81.5
1972	79.9	<**	82.2	106.1	=	107.2	86.3	=	88.3
1973	75.5	=	75.1	94.4	<***	97.6	84.1	=	85.4
1975	80.6	>***	73.6	92.4	<**	94.7	86.2	>**	83.7
1976	80.5	>***	71.5	97.9	>***	88.0	89.2	>***	79.7
1978	84.0	=	83.5	98.7	=	100.1	86.1	=	85.9
1979	78.1	<***	80.6	97.4	=	96.3	81.3	<***	83.2
1981	87.3	>***	82.5	100.8	<*	104.2	89.7	>***	86.4
1982	86.8	=	86.9	105.8	>	100.1	90.0	=	89.1
1983	90.8	>***	88.1	98.0	<*	103.1	91.6	>***	88.1
1984	90.4	>***	84.2	102.3	=	102.5	92.5	>***	87.5
1985	91.0	=	92.0	101.2	=	105.6	92.2	<*	93.6
1986	91.8	>***	85.6	106.8	=	105.0	92.9	>***	87.0
1987	93.7	=	95.4	111.6	=	114.1	96.9	=	98.7

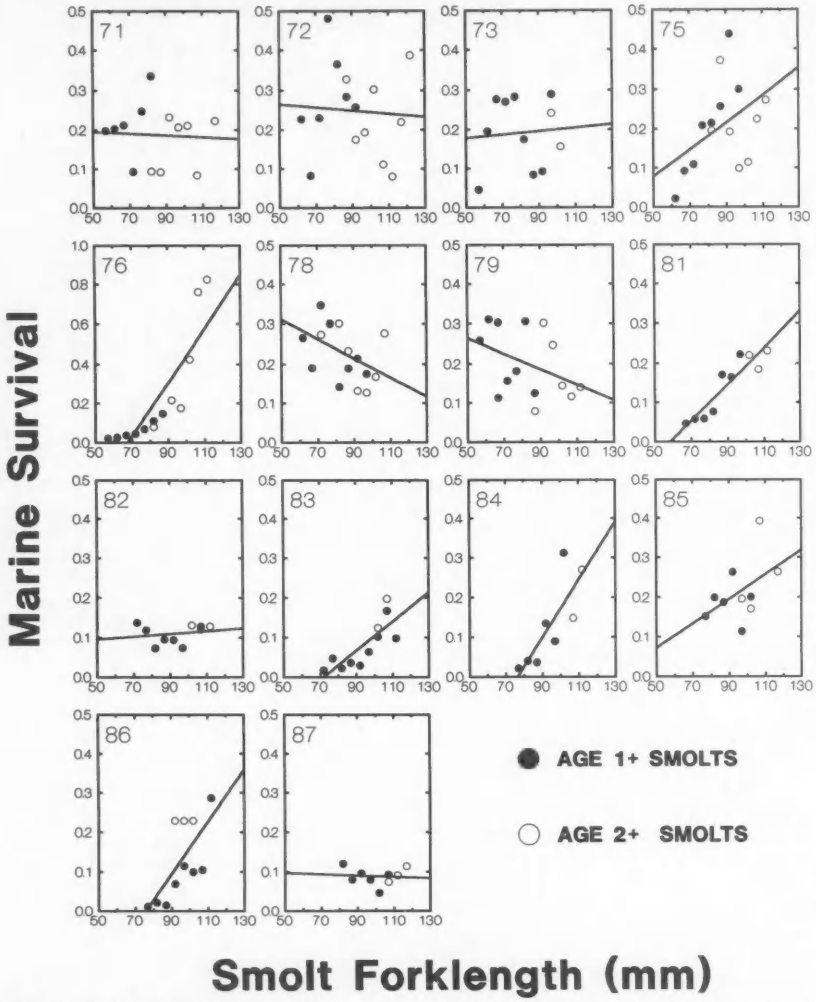

FIG. 7. Marine survivals vs. smolt forklength in 5 mm FL classes. Regression lines shown were fitted to survival data for both freshwater age groups.

coastal upwelling at 51°N, 131°W, over the period June through August inclusive. We hypothesized that relatively strong upwelling coupled with onshelf transport of upwelled water indicated by high salinities and low temperatures near shore should have indicated favourable growth conditions for juvenile salmon off the west coast of Vancouver Island north of Barkley Sound. For both the spring and summer environmental time series, the averaging periods correspond to the approximate location of smolts migrating northward along the coast of Vancouver Island to rearing areas off the northwest coast of Vancouver Island.

Data Relevant to the Predation Intensity Hypothesis

If variation in predation intensity was an important factor in determining interannual variation in coho salmon survival then ecologically similar species of the same size and resident on the continental shelf should have been similarly affected. In Barkley Sound, juvenile coho salmon are often found associated with schools of the considerably more abundant Pacific herring (*Clupea harengus pallisi*; K. Groot, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, pers. comm.). Age 1 and 2 herring and coho salmon in thir first ocean summer are approximately the same size and probably have similar diets (Hourston 1959; Healey 1976, 1978). Thus, juvenile coho salmon may be ecologically similar to herring over their first few months in the ocean. Pacific herring are an important prey species of the major piscivorous fish on the west coast, and in particular Pacific cod (*Gadus macrocephalus*, Walters et al. 1986) and Pacific hake (*Merluccius productus*, Ware and McFarlane 1988).

Changes in the abundance of Pacific herring off the west coast of Vancouver Island are relevant to our thesis because it is believed that declines in herring numbers since the mid-1970's were the result of increased predation pressure (Ware and McFarlane 1988). Predation pressure has increased either

TABLE 2. Regression of marine smolt survivals in 5 mm FL intervals on the mid-points of the intervals. r^2 has been adjusted for the degrees of freedom. P is the probability that the slope ($\hat{\beta}_{survival}$) is different from zero.

	Age 1⁺				Age 2⁺				Overall			
Smolt year	$\hat{\beta}_{survival}$	n	P	$r^2_{adjusted}$	$\hat{\beta}_{survival}$	n	P	$r^2_{adjusted}$	$\hat{\beta}_{survival}$	n	P	$r^2_{adjusted}$
1971	0.0040	6	0.34	0.032	0.0024	7	0.36	0.002	−0.0002	13	0.86	0.0
1972	0.0045	7	0.38	0.0	0.0003	8	0.94	0.0	−0.0004	15	0.83	0.0
1973	0.0006	9	0.82	0.0	—	2	—	—	0.0005	11	0.82	0.0
1975	0.0098	8	0.002	0.81	−0.0010	7	0.81	0.0	0.0034	15	0.10	0.14
1976	0.0041	7	0.002	0.86	0.0269	6	<0.01	0.81	0.0136	13	0.71	<0.001
1978	−0.0027	8	0.23	0.10	−0.0030	8	0.21	0.13	−0.0024	16	0.15	0.08
1979	−0.0030	8	0.39	0.0	−0.0021	6	0.67	0.0	−0.0020	14	0.09	0.16
1981	0.0061	7	0.002	0.85	—	3	—	—	0.0046	10	<0.001	0.83
1982	−0.0004	7	0.68	0.0	—	3	—	—	0.0004	10	0.58	0.0
1983	0.0029	9	0.009	0.60	—	2	—	—	0.0037	11	0.003	0.60
1984	0.0098	6	0.041	0.61	—	2	—	—	0.0073	8	0.013	0.62
1985	0.0004	6	0.90	0.0	0.0050	4	0.57	0.0	0.0031	10	0.16	0.13
1986	0.0063	8	0.007	0.69	—	3	—	—	0.0066	11	0.016	0.44
1987	−0.0015	6	0.25	0.14	—	3	—	—	−0.0002	9	0.82	0.0

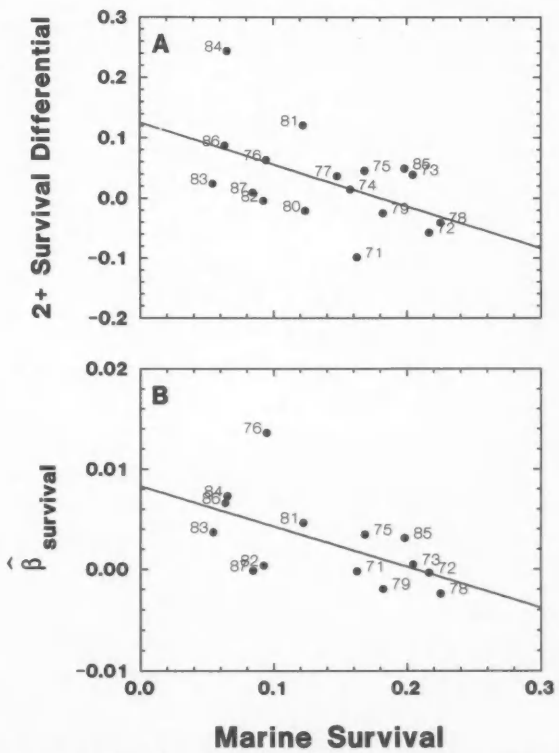

FIG. 8. (A) 2^+ survival differential and (B) $\hat{\beta}_{survival}$ (both age groups) vs. marine survival for both freshwater age groups combined. Fitted lines are regression models 1 and 2, Table 3.

because of northward range extensions of hake associated with rising ocean temperatures, or because of increased abundances or predation rates of hake and other piscivorous fishes. Furthermore, Ware and McFarlane have calculated that the biomass of piscivorous fishes was sufficient to have accounted for all of the annual mortality in age 1 and older herring. Although salmon smolts are not known to be an important prey species of the above fish (e.g. Outram and Haegele 1972), the relative scarcity of juvenile salmon compared to herring would make it unlikely that salmon would constitute a significant fraction of any of the above piscivores' diets. Even if juvenile salmon were an incidental prey species, there was sufficient predator biomass to have caused substantial mortality of Carnation Creek coho salmon smolts.

We therefore compared the marine survivals of smolts with an index of age 1 and 2 herring survivals derived from the data of Haist et al. (1987) and Haist and Schweigert (1990). Age-specific herring abundances for the southwest coast of Vancouver, including Barkley Sound, were estimated using an age-structured model (Fournier and Archibald 1982; Stocker et al. 1983; Haist and Schweigert 1990). The abundance of age 1 herring was not measured but was back-calculated from measured abundances of age 2 and older herring using assumed constant natural survival rates. Consequently, survival rates of age 1 and 2 herring fluctuate around the assumed natural survival rate and must be considered as an index of survival only. The predation-intensity hypothesis would be supported if herring and salmon survival rates covaried.

Results

Importance of Smolt Size

Prior to clear-cut logging in the watershed (pre-1977 smolt year), Carnation Creek produced roughly equal numbers of age 1^+ and age 2^+ smolts (Fig. 4A). After 1977, the numbers of age 2^+ smolts steadily declined, and from 1978 to 1987 at least 90% of the smolts were aged 1^+. The 2^+ smolts were, on average, longer and heavier than the 1^+ smolts (Fig. 4B).

If smolt size was an important determinant of early marine survival and assuming that the two age-groups of smolts differed only in size, then 2^+ smolts should have consistently survived better than 1^+ smolts compared within smolt years. Although, on average, marine survivals of the 2^+ smolts (average: 16.6%; range: 7.7–29.9%) were greater than those of 1^+ smolts (average: 13.8%; range: 5.3–23.4%; Fig. 5A), the difference was not statistically significant (paired t-test: $t = -1.52$, $df = 16$, $P = 0.15$). The proportion of 1^+ fish in the adults (average 0.730) was not significantly different from the same proportion in the smolts (average 0.752; paired t-test: $t = 1.33$, $df = 16$, $P = 0.17$; Fig. 5B). In addition, there was no significant

TABLE 3. Regression models referred to in text. P is the probability that the slope is different from zero. $\Delta d_{circulus}$ is the average inter-circular spacing over the first five ocean circuli, and is a measure of early ocean growth rate. SSSal is the mean sea-surface salinity at Kains Island from June through August.

Regression model	$r^2_{adjusted}$	P	n
1. 2^+ survival differential $= 0.125 - 0.697 \times$ marine survival	0.21	<0.05	17
2. $\beta_{survival} = 0.00828 - 0.0403 \times$ marine survival	0.23	<0.05	14
3. 2^+ survival differential $= 0.137 - 0.787 \times$ age 1^+ marine survival	0.37	<0.01	17
4. $\beta_{survival} = 0.00840 - 0.0413 \times$ age 1^+ marine survival	0.3	0.01	14
5. Age 1^+ marine survival $= -0.838 + 26.3 \times \Delta d_{circulus} 1^+$(mm)	0.77	<0.0001	14
6. Age 2^+ marine survival $= 0.174 - 0.101 \times \Delta d_{circulus} 2^+$(mm)	0.00	≈1	14
7. Marine survival $= -0.695 + 22.0 \times \Delta d_{circulus}$(mm)	0.73	<0.0001	14
8. 2^+ survival differential $= 0.908 - 23.1 \times \Delta d_{circulus}$(mm)	0.41	<0.01	14
9. $\beta_{survival} = 0.0439 - 1.09 \times \Delta d_{circulus}$(mm)	0.33	<0.05	14
10. 2^+ survival differential $= 0.947 - 24.6 \times \Delta d_{circulus} 1^+$(mm)	0.42	<0.01	14
11. $\beta_{survival} = 0.047 - 1.20 \times \Delta d_{circulus} 1^+$(mm)	0.37	<0.05	14
12. $\Delta d_{circulus}$(mm) $= 0.00445 + 0.000368 \times$ FL (mm)	0.40	<0.0001	877
13. Marine survival $= -1.86 + 0.0636 \times$ SSSal(‰)	0.59	0.0005	17
14. $\Delta d_{circulus}$(mm) $= -0.0364 + 0.00236 \times$ SSSal(‰)	0.47	0.005	14

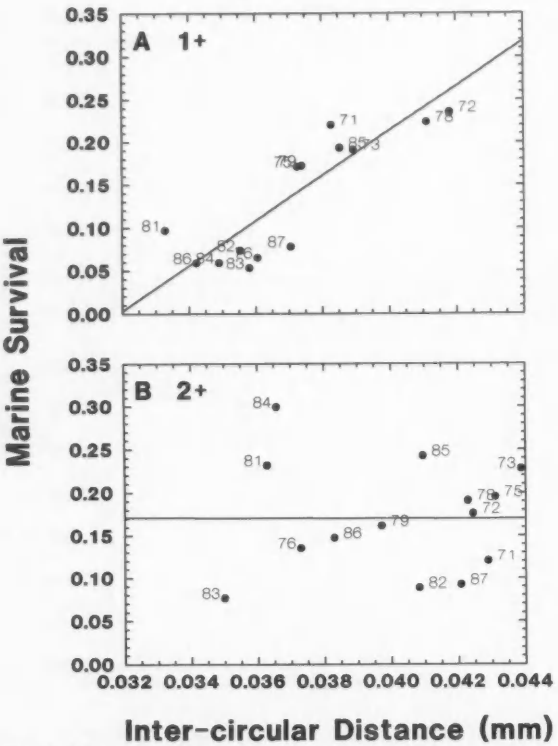

FIG. 9. Marine survival of (A) age 1^+ and (B) age 2^+ smolts vs. average inter-circular distance. Fitted lines are regression models 5 and 6, Table 3.

relationship between marine survival and smolt size ($r = 0.162$, $n = 34$, $P = 0.36$; Fig. 6).

We could not fully test our assumption that age 1^+ smolts differed only in size from 2^+ smolts, but we did examine two factors which could have contributed to different marine survivals, independent of smolt size. First, emigration dates could have been different for the two age-groups, leading to different marine survivals (Bilton et al. 1982). Holtby et al. (1989) found that the median date of age 1^+ smolt emigration tended to be 0–6 d before the median data for age 2^+ smolts from 1971 to 1977. From 1978 to 1987 the median dates were on average the same. In view of the inter-quartile range in emigration date of 12–31 d, differences of a few days in the median emigration dates of the two age-groups were probably of no significance. Second, age 2^+ smolts may have grown more slowly in the ocean than age 1^+ smolts since they had grown more slowly in freshwater. Slower growth in the ocean might have diminished any advantage to ocean survival of larger initial size. We used average intercircular distance over the first five ocean circuli to compare early ocean growth rates of the two age-groups of smolts. The average inter-circular distance for age 2^+ smolts (0.0401 mm) was significantly greater than for 1^+ smolts (0.0371 mm; paired t-test: $t = 5.91$, df = 13, $P < 0.001$). We think that it was reasonable to assume that the principal difference between the two age-groups was average size.

If smolt survival was not size-dependent then the mean of the smolt size-frequency distribution back-calculated from the adult scales should have been the same as the mean of the size-frequency distribution observed for emigrating smolts. Expected and observed mean smolt sizes were compared in 14 yr (Table 1). For both smolt age-groups combined, small smolts were significantly over-represented in the return in 2 yr, in 6 yr there were no significant differences between the observed and expected mean sizes, and in the remaining 6 yr small smolts were significantly under-represented in the return. Similar differences between expected and observed mean sizes were found for the age 1^+ smolts considered alone (Table 1), but for the age 2^+ smolts the observed mean smolt size was generally equal to or less than the predicted mean size (Table 1).

To examine the effects of smolt size on survival within years in more detail, we calculated smolt survivals within 5 mm intervals of estimated smolt FL. We first compared survivals of age 1^+ and age 2^+ smolts in those intervals where both occurred and found no significant difference in survival rate (mean difference = 0.00224, age 1^+ survival greater; paired t-test: $t = 0.097$, df = 25, $P > 0.9$). Since smolts of the same size but of different age survived at the same rate, we used smolts of both ages in regressions of survival on smolt FL. (This result is further evidence of the validity of our assumption that smolts of different ages differed importantly only in size.) In 10 of 14

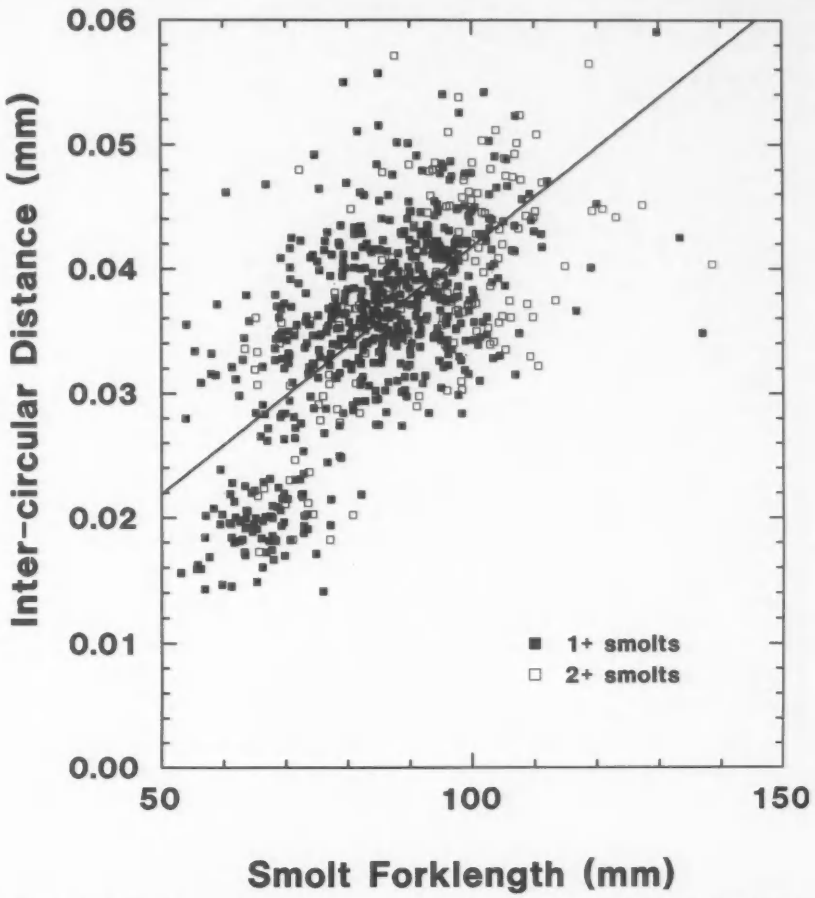

FIG. 10. Average inter-circular distance vs. smolt FL for the 14 smolt years of Table 1. Fitted line is from regression model 12, Table 3.

yr there was no significant relationship between size and survival (Fig. 7; Table 2). In the other 4 yr, large smolt size conferred a significant survival advantage. If only the age 1^+ smolts were used in the regression, then $\beta_{survival}$ was significantly greater than zero in six of 14 yr (Table 2). In those same years, mean smolt lengths back-calculated from adult scales were significantly greater than those expected from smolt length distributions (Table 1). In several years (e.g. 1975, 1976, 1983) a sigmoidal or saturating function of size would have been more appropriate than a linear model. However, we used linear models so that we could use the slope of the line ($\beta_{survival}$) as an indicator of the relative survival advantage of large smolt size.

Our two measures of the relative survival advantage of large smolt size (2^+ survival differential and $\beta_{survival}$) were significantly correlated ($r = 0.66$; $n = 14$; $P = 0.011$). Years in which large size was of advantage to survival tended to be the years in which age 2^+ smolts had a survival advantage.

The advantage to smolt survival of large size was inversely related to overall survival. Both the 2^+ survival differential and $\beta_{survival}$ varied inversely with smolt to adult survival (Fig. 8; models 1 and 2 of Table 3). As overall survival fell, the 2^+ smolts survived increasingly better than the 1^+ smolts and large smolts survived better than small regardless of freshwater age. Both relationships were stronger when the survival of the age 1^+ smolts rather than overall survival was used (models 3 and 4 of Table 3), indicating that both relationships were being driven by variation in the survival of age 1^+ smolts.

Early Ocean Growth

Growth rates in the 2 mo after ocean entry were positively correlated with the marine survivals of 1^+ smolts (Fig. 9A) and of 1^+ and 2^+ smolts combined (models 5–7 of Table 3). The rate of growth was measured by the average inter-circular spacing over the first five circuli. The correlation between inter-circular distance and survival was strongest if only the age 1^+ smolts were considered (model 5 of Table 3). The survival of the age 2^+ smolts was not significantly correlated with inter-circular distance (model 6 of Table 3). Both the 2^+ survival differential and $\beta_{survival}$ were negatively correlated with the inter-circular spacing (models 8 and 9 of Table 3; Fig. 10). The latter correlations were stronger when the inter-circular distance of only the age 1^+ smolts was used (models 10 and 11 of Table 3), an indication that these relationships were being driven by variations within the younger, and consequently smaller, smolts.

TABLE 4. ANOVAs for effects of freshwater age and smolt FL on inter-circular distance ($\Delta d_{circulus}$).

ANOVA dependent variable: $\Delta d_{circulus}$; categorical variable: freshwater age; covariate: smolt forklength

Source	SS	df	MS	*F*	*P*
Smolt forklength	0.0219	1	0.0219	555.6	<0.0001
Freshwater age	0.00002	1	0.00002	0.403	0.53
Error	0.0344	874	0.000039		

ANOVA dependent variable: $\Delta d_{circulus}$; categorical variable: smolt year; covariate: smolt forklength

Source	SS	df	MS	*F*	*P*
Smolt year	0.0131	13	0.00101	40.8	<0.0001
Smolt forklength	0.0076	1	0.0076	307.6	<0.0001
Error	0.0213	862	0.000025		

Post-hoc comparison of $\Delta d_{circulus}$ corrected for smolt length between years of low survival (<0.10) and high survival.

Source	SS	df	MS	*F*	*P*
Comparison	0.0053	1	0.0053	214.6	<0.0001
Error	0.0213	862	0.000025		

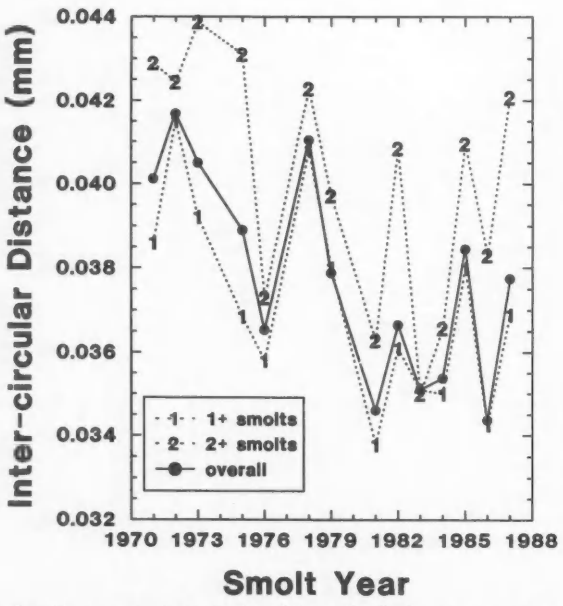

FIG. 11. Average inter-circular distance by freshwater age group against smolt year.

Inter-circular distances were positively correlated with smolt length at ocean entry (model 12 of Table 3; Fig. 10). This relationship underlies the significantly higher average growth rates of the age 2^+ smolts compared to the age 1^+ smolts noted earlier. To confirm this we did an analysis of covariance of inter-circular distance with freshwater age as the grouping variable and smolt FL as the covariate, and found no significant effect of age (Table 4). Because smolt size changed considerably over the study period (Fig. 4B), it was possible that the correlation between smolt survival and growth rate was a spurious result of a simultaneous, but coincidental, increase in both survival and smolt size following 1977. However, relatively poor survivals (Fig. 5A) and slow growth (Fig. 11) occurred often in the 1980's while at the same time smolt size was relatively large (Fig. 4B). To further confirm that growth rate and survival were positively associated, independent of smolt size, we did an analysis of covariance on average inter-circular distance with smolt year as the categorical variable and smolt forklength as the covariate (Table 4). A post-hoc comparison between years of low (<10%) and high (≥10%) survival (Table 4) confirmed that inter-circular distance differed significantly in years of low and high survival, independently of smolt size.

Density-Dependence

We could find no indication that smolt numbers affected either smolt survival or early ocean growth. Marine survival was not correlated with either the number of smolts leaving Carnation Creek (age 1^+: $r=-0.37$, $P>0.1$, $n=17$; age 2^+: $r=-0.29$, $P>0.25$, $n=17$), or with the number of coho smolts estimated to have entered the Sound ($r=-0.31$, $P>0.25$, $n=17$). Similarly, the average inter-circular distance was not significantly correlated with either the number of smolts leaving Carnation Creek (1^+: $r=-0.42$, $P>0.1$, $N=14$; 2^+:$r=0.49$, $P=0.08$, $n=14$) or the estimated regional production ($r=-0.46$, $P>0.1$, $n=14$). We were unable to detect density-dependent effects on survival and growth when the analysis was restricted to the 6 yr when overall smolt survival was less than 10% (smolt years 1976, 1982–1984, 1986, and 1987).

Ocean Conditions

The six environmental time series were selected as probable indicators of upwelling conditions at times and locations appropriate to the migration of coho salmon smolts up the west coast of Vancouver Island. Within both time period-locations, sea-surface salinities and temperatures were negatively correlated and sea-surface salinities and coastal upwelling indices were positively correlated (Table 5), but none of the correlations was statistically significant. The sign of the correlation between sea-

TABLE 5. Product–moment correlations among the six environmental time series. SST is sea-surface temperature (°C), SSSal is sea-surface salinity (‰), and CUI is the coastal upwelling index. Summer values were averages from June through August. For all correlations n was 17. Significant departure from $r = 0$: $^{**}P<0.01$; $^{***}P<0.001$.

Environmental variable	1.	2.	3.	4.	5.
1. May SST Amphitrite Point	—				
2. May SSSal Amphitrite Point	−0.111	—			
3. May CUI 48°N, 125°W	0.227	0.341	—		
4. Summer SST Kains Island	0.801***	−0.210	0.389	—	
5. Summer SSSal Kains Island	−0.316	0.607**	−0.091	−0.339	—
6. Summer CUI 51°N, 131°W	0.009	0.181	0.069	−0.079	0.327

TABLE 6. Relationships between marine survival and inter-circular distance with six environmental time series. Table entries are Pearson correlation coefficients, r. E_{sign} is the expected sign of r. For H_0: $r=0$, $^{*}P<0.1$, $^{**}P<0.05$, $^{***}P<0.01$, $^{****}P<0.001$.

		Marine survival ($n=17$)			Δcirculus ($n=14$)		
Environmental variable	E_{sign}	Age 1^+	Age 2^+	Both ages	Age 1^+	Age 2^+	Both ages
May SST Amphitrite Point	−	−0.31	−0.14	−0.30	−0.27	−0.54**	−0.38
May SSSal Amphitrite Point	+	0.49**	−0.29	0.51**	0.53*	0.64***	0.61***
May CUI 48°N, 125°W	+	0.02	−0.70***	−0.13	0.05	0.11	0.05
Summer SST Kains Island	−	−0.21	−0.27	−0.31	−0.19	−0.57**	−0.34
Summer SSSal Kains Island	+	0.75***	0.16	0.79***	0.70***	0.76****	0.72***
Summer CUI 51°N, 131°W	+	0.22	−0.11	0.25	0.24	0.43*	0.25

surface temperature and the coastal upwelling index was different in the two time period-locations (Table 5), indicating that mid-year sea-surface conditions were similar over the northwest coast of Vancouver Island.

For both survival and inter-circular distance we calculated 12 correlations (two age-groups × six environmental time series) and for each determined an expected direction based on the hypotheses previously outlined. For marine survivals nine of the 12 comparisons were of the expected sign and three were significant ($P<0.05$; Table 6). Assuming that values of r, the correlation coefficient, were randomly distributed around zero, the probability of finding 10 of 12 correlations with the expected sign was 0.075. Similarly, for inter-circular distances all 12 correlations were of the expected sign, an occurence with a probability of less than 0.001. Five of the 12 were statistically significant (Table 6). For both survival and growth the strongest and most consistent environmental correlate was the summer sea-surface salinity at Kains Island, near the northwest tip of Vancouver Island (models 13 and 14 of Table 3). In summary, relatively fast growth and high survival were associated with ocean conditions indicative of a strong upwelling along the northwest coast of Vancouver Island.

Although we used linear models to quantify the relationships between smolt survival, early ocean growth, and ocean conditions, linear models may not have been appropriate. For both survival and early ocean growth, a threshold model with two steps might have better described the observations (Fig. 12A, B). In such a model the dependent variable varies around one of two average values and jumps between the two values when a threshold value of some driving variable (or its correlate) is crossed. For Carnation Creek coho, overall survival fluctuated around values of ≈0.088 and ≈0.18 with a threshold summer salinity value of ≈31.5‰ (Fig. 12A). Similarly, the average inter-circular distance fluctuated around values of ≈0.036 mm and ≈0.40 mm with the same threshold summer salinity value (Fig. 12B).

Survival Variations in Age 1 and 2 Pacific Herring

Changes in the abundance of Pacific herring off the southwest coast of Vancouver Island since 1970 have been ascribed to changes in predation intensity (Ware and McFarlane 1988). Indexed survival rates of age 1 and 2 herring and the marine survival of Carnation Creek coho were positively correlated ($r=0.6$, df = 15, $P<0.01$; Fig. 13) over our study period. Since age 1 and 2 herring and coho salmon smolts are similarly sized, are ecologically similar, and co-occur in Barkley Sound, it seems reasonable to infer that the covariation in their survival rates was not coincidental.

Discussion

The three general hypotheses explaining variations in the marine survival of coho salmon, "ocean-productivity," "density–dependence," and "predation–intensity," present two alternative views of the marine life histories of coho salmon. The "ocean–productivity" and "density–dependence" hypotheses both assume that the rate of predation on juvenile salmon depends on their initial size and on their growth rate in the ocean, and stems from a relatively constant number of predators. Both initial size and growth rate determine the length of time the juvenile salmon remain below some critical size threshold above which predation lessens. The two hypotheses differ in the mechanism that produces variations in the duration of maximum susceptibility. In contrast, the "predation–intensity" hypothesis as formulated by Fisher and Pearcy (1988) assumes that varying levels of size-independent predation accounts for variations in ocean survival.

We found no indication that either the survival or growth of coho salmon leaving Carnation Creek varied with numbers of juvenile coho salmon estimated to have entered Barkley Sound, even though increased hatchery production resulted in an approximate 2.4× increase in total coho numbers through the 1970's and early 1980's. The numbers of ecologically similar

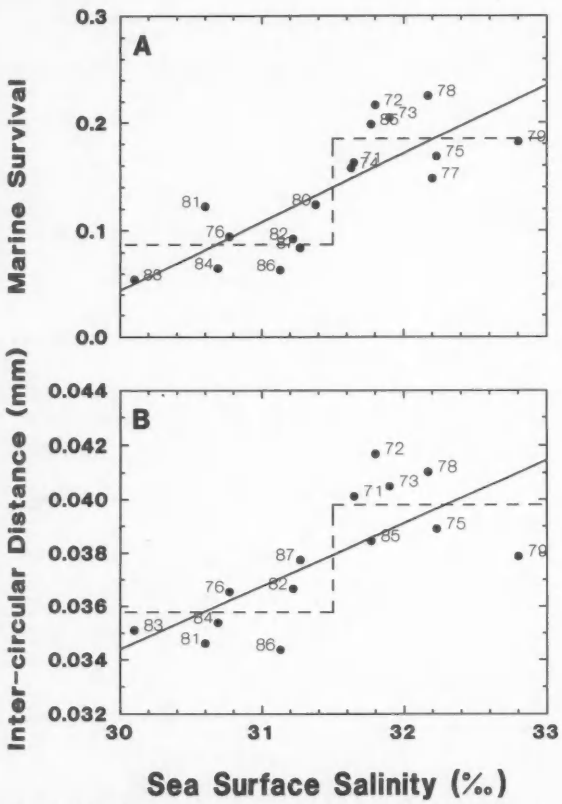

FIG. 12. (A) Marine survival both age groups combined and (B) average inter-circular distance, both age groups combined versus summer sea-surface salinity at Kains Island. Dashed lines represent possible stepped models.

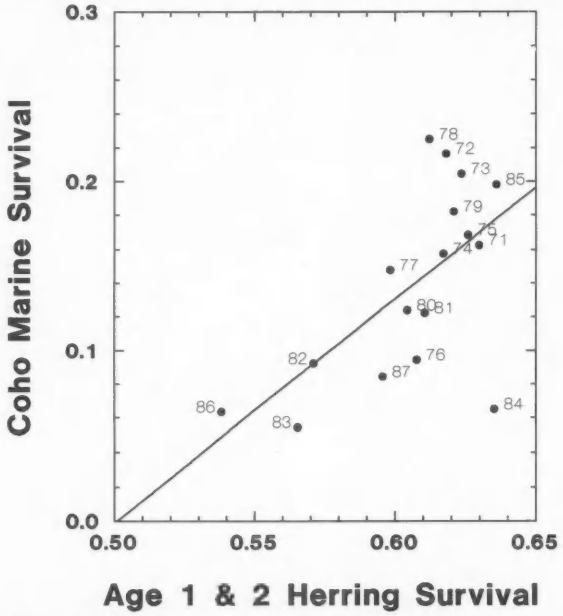

FIG. 13. Marine smolt survival of Carnation Creek coho salmon versus index of age 1 and 2 Pacific herring survival off southwest coast of Vancouver Island from 1971 to 1987.

marine species in and around Barkley Sound were probably far greater than the numbers of juvenile salmonids. For example, $\approx 1.1 \times 10^9$ age 1 and 2 Pacific herring were estimated to have resided in Barkley Sound and the neighboring coastal area of Vancouver Island (Haist et al. 1987). The total number of young herring off southern Vancouver Island has fallen substantially in the last two decades (Haist et al. 1987), suggesting that densities of small pelagic fishes, ecologically similar to coho salmon, were declining over our study period and were lowest in the same years that marine survivals of coho salmon were poorest. Consequently, within the estimated densities observed over the years of our study, we reject the density–dependent hypothesis as an explanation for variations in coho smolt survival at Carnation Creek.

Although large smolt size did not confer a consistent survival advantage among smolts leaving Carnation Creek, either between or within years, large smolts did have a measurable survival advantage in years when overall marine survivals were poor. Marine survival and early ocean growth were positively associated. One explanation of both observations would be that size-dependent mortality was a determinant of inter-annual variability in the marine survival of coho salmon smolts leaving Carnation Creek. Growth rates during early sea life determined the length of time that fish remained small and, presumably, most susceptible to predation. During years when growth rates were particularly slow, mortality was high. It was only in years when mortality rates were high that the survival advantage associated with large size (superior escape ability, faster growth, and a shorter time to attain a size-threshold) became sufficiently large to be detected using our methods.

Thus, the "predation–intensity" hypothesis of Fisher and Pearcy (1988) is inconsistent with our observations. Whereas they found no evidence for either size-dependent mortality or for differences in growth rate between low and high survival years, we found considerable evidence that smolt size and early ocean growth were predictive of smolt survival. We note, however, that the average smolt sizes reported by Fisher and Pearcy (1988) ranged between 149–162 mm, 1.9 × the average size of 1^+ smolts and 1.6 × the size of 2^+ smolts observed at Carnation Creek. Assuming an average growth rate of 0.93 $mm \cdot d^{-1}$ (Fisher and Pearcy 1988), 2^+ smolts leaving Carnation Creek would have required 60 d to reach 150 mm. Using the same growth rate, the fifth ocean circulus was deposited at an average size of 145 mm, ≈45–51d after leaving Carnation Creek. We suspect that Fisher and Pearcy did not observe consequences of either size-dependent mortality or growth because smolts produced by Oregon hatcheries were larger than the size threshold where such size-dependent processes were operative.

The observations that we have presented indicate that variability in the marine survival of Carnation Creek coho salmon was closely related to ocean conditions off the west coast of Vancouver Island over the first 4 mo of ocean life. Conditions favourable to upwelling and subsequent transport of the upwelled water onto the continental margin may have been conducive to fast growth for juvenile salmon migrating up the coast. Relatively fast growth was in turn associated with relatively high survival. Thus, our observations support the "ocean–productivity" hypothesis.

The covariation of survivals of coho salmon smolts and Pacific herring suggest some common underlying factor. Since declines in herring abundance have been linked to changes in the distribution and abundance of a piscivorous fish (probably Pacific hake) around Barkley Sound (Ware and McFarlane 1988), the common factor may have been variations in predation intensity. A large and transient stock of Pacific hake arrive in the Barkley Sound region from the south in May. The northward and inshore movements of these fish are influenced by conditions around the time of the spring transition in the Vancouver Island Coastal Current (Smith et al. 1990). Early and abrupt transitions not only produce conditions favourable to upwelling along the coast but also delay the formation of, and weaken, the northward flowing Coastal Current, possibly delaying the northward movements of hake. In years of late or weak transitions southerly surface waters, and probably hake as well, move more strongly northward along the coast at the same time as migrating coho salmon smolts (Smith et al. 1990).

We propose that variations in the smolt survivals of Carnation Creek coho salmon were caused by changes in near-shore ocean productivity that affected salmon growth rates and consequently their susceptibility to predation, and by simultaneous changes in predation intensity caused by changes in some combination of predator distribution, abundance, or type. Further resolution of the mechanisms underlying variation in coho salmon smolt survival at Carnation Creek cannot be made from input or output data like ours, but must depend on the collection of observational data during the first months of sea life.

Acknowledgments

We thank the many people who have participated in the Carnation Creek study over the years. We thank W. G. Pearcy for suggesting this inquiry, M. Healey, J. Schweigert, D. Blackbourn, R. Tanasichuk, D. Ware, D. L. Scarnecchia, and an anonymous reviewer, who provided many helpful criticisms of draft manuscripts and B. Smith who kindly provided us with a reprint of his manuscript on hake movements.

References

ANDERSEN, B. C. 1983. Fish populations of Carnation Creek and other Barkley Sound streams 1970–1980. Can. Data Rep. Fish. Aquat. Sci. 415: 267 p.

1984. Fish populations of Carnation Creek and other Barkley Sound streams 1981–1982. Can. Data Rep. Fish. Aquat. Sci. 435: 63 p.

1985. Fish populations of Carnation Creek and other Barkley Sound streams 1983–1984. Can. Data Rep. Fish. Aquat. Sci. 553: 62 p.

1987. Fish populations of Carnation Creek and other Barkley Sound streams 1985–1986. Can. Data Rep. Fish. Aquat. Sci. 657: 59 p.

Argue, A. W., L. M. Patterson, and R. W. Armstrong. 1970. Trapping and coded-wire tagging of wild coho, chinook and steelhead juveniles from the Cowichan–Koksilah River system, 1976. Fish Mar. Serv. Tech. Rep. 850: 117 p.

ARGUE, A. W., M. P. SHEPPARD, T. F. SHARDLOW, AND A. D. ANDERSON. 1987. A review of the troll fisheries in southern British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1502: 150 p.

ARMSTRONG, R. W., AND A. W. ARGUE. 1977. Trapping and coded-wire tagging of wild coho and chinook juveniles from the Cowichan River system, 1975. Fish. Mar. Serv. Tech. Rep. Ser. Pac/T-77-14: 58 p.

BAKUN, A. 1973. Coastal upwelling indices, West coast of North America, 1946–71. NOAA Tech. Rep. NMFS SSRF-671: 103 p.

BARANSKI, C. 1989. Coho smolt production in ten Puget Sound streams. Wash. Dep. Fish. Tech. Rep. 99: 29 p.

BILTON, H. T., D. F. ALDERDICE, AND J. T. SCHNUTE. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Can. J. Fish. Aquat. Sci. 39: 426–447.

BROWN. T. G., I. V. WILLIAMS, AND A. LANGSTON. 1987. Watershed data base: Barkley Sound, Vancouver Island. Can. Data. Rep. Fish. Aquat. Sci. 667: 143 p.

CHAMBERLAIN, T. W. [ed.] 1987. Proceedings of the Workshop: applying 15 years of Carnation Creek results. Carnation Creek Steering Committee, c/o Pacific Biological Station, Nanaimo, B.C. V9R 5K6: 239 p.

CLUTTER, R. I., AND L. E. WHITESEL. 1956. Collection and interpretation of sockeye salmon scales. Int. Pac. Salmon Fsh. Comm. Bull. 9: 159 p.

FISHER, J. P., AND W. G. PEARCY. 1988. Growth of juvenile coho salmon (*Oncorhynchus kisutch*) off Oregon and Washington, USA, in years of differing coastal upwelling. Can. J. Fish. Aquat. Sci. 45: 1036–1044.

FOERSTER, R. E. 1954. On the relation of adult sockeye salmon (*Oncorhynchus nerka*) returns to known smolt seaward migrations. J. Fish. Res. Board Can. 11: 339–350.

FOURNIER, D., AND C. P. ARCHIBALD. 1982. A general theory for analyzing catch at age data. Can. J. Fish. Aquat. Sci. 39: 1195–1207.

GIOVANDO, L. F. 1985. Observations of seawater temperature and salinity at British Columbia shore stations, 1984. Can. Data Rep. Hydogr. Ocean Sci. 41: 102 p.

HAIST, V., AND J. F. SCHWEIGERT. 1990. Stock assessments for British Columbia herring in 1989 and forecasts of the potential catch in 1990. Can. MS Rep. Fish. Aquat. Sci. 2049: 62 p.

HAIST, V., J. F. SCHWEIGERT, AND D. FOURNIER. 1987. Stock assessments for British Columbia Herring in 1986 and forecasts of the potential catch in 1987. Can. MS Rep. Fish. Aquat. Sci. 1929: 63 p.

HEALEY, M. C. 1976. Herring in the diets of Pacific salmon in Georgia Strait. Fish. Res. Board Can. MS Rep. Ser. 1382: 37 p.

1978. The distribution, abundance, and feeding habits of juvenile Pacific salmon in Georgia Strait, British Columbia. Fish. Mar. Serv. Tech Rep. 788: 49 p.

1982. Timing and relative intensity of size-selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can. J. Fish. Aquat. Sci. 39: 952–957.

HOLTBY, L. B. 1988. Effects of logging on stream temperatures in Carnation Creek, British Columbia, and associated impacts on the coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 45: 502–515.

HOLTBY, L. B., AND G. F. HARTMAN. 1982. The population dynamics of coho salmon (*Oncorhynchus kisutch*) in west coast rain forest stream subjected to logging, p. 308–347 *In* G. F. Hartman [ed.] Proceedings of the Carnation Creek workshop a 10-year review. (Available from Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6 Canada)

HOLTBY, L. B., G. F. HARTMAN, AND J. C. SCRIVENER. 1984. Stream indexing from the perspective of the Carnation Creek experience, p. 87–111. *In* P. E. K. Symons and M. Waldichuk [ed.] Proc. of the Workshop on Stream Indexing for Salmon Escapement Estimation. Can. Tech. Rep. Fish. Aquat. Sci. 1326: 258 p.

HOLTBY, L. B., T. E. MCMAHON, AND J. C. SCRIVENER. 1989. Stream temperatures and inter-annual variability in the emigration timing of coho salmon (*Oncorhynchus kisutch*) smolts and fry and chum salmon (*O. keta*) fry from Carnation Creek, British Columbia. Can. J. Fish. Aquat. Sci. 46: 1396–1405.

HOLTBY, L. B., AND J. C. SCRIVENER. 1989. Observed and simulated effects of climatic variability, clear-cut logging and fishing on the numbers of chum salmon (*Oncorhynchus keta*) and coho salmon (*O. kisutch*) returning to Carnation Creek, British Columbia, p. 62–81. *In* C. D. Levings, L. B. Holtby, and M. A. Henderson [ed.] Proceedings of the national workshop on effects of habitat alteration on salmonid stocks. Can. Spec. Publ. Fish. Aquat. Sci. 105: 199 p.

HOURSTON, A. S. 1959. Effects of some aspects of environment on the distribution of juvenile herring in Barkley Sound. J. Fish. Res. Board Can. 16: 283–308.

MASON, J. E., AND A. BAKUN. 1986. Upwelling index update, U.S. west coast, 33N-48N latitude. NOAA Tech. Memo NOAA-TM-NMFS-SWFC-67: 81 p.

MATHEWS, S. B., AND R. BUCKLEY. 1976. Marine mortality of Puget Sound coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 33: 1677–1684.

NICKELSON, T. E. 1986. Influences of upwelling, ocean temperature, and smolt abundance on marine survival of coho salmon (*Oncorhynchus kisutch*) in the Oregon Production Area. Can. J. Fish. Aquat. Sci. 43: 527–535.

OUTRAM, D. N., AND C. W. HAEGELE. 1972. Food of Pacific hake (*Merluccius productus*) on an offshore bank southwest of Vancouver Island, British Columbia. J. Fish. Res. Board Can. 29: 1792–1795.

PARKER, R. R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fish. Res. Board Can. 28: 1503–1510.

PETERMAN, R. M. 1978. Tesing for density-dependent marine survival in Pacific salmonids. Can. J. Fish. Aquat. Sci. 35: 1434–1450.

RANKIS, A. E. 1987. Factors biasing scale analysis estimates of size selective mortality in Columbia River coho salmon during rearing density experiments. M.Sc. thesis, University of Washington, Seattle, WA. 181 p.

SCARNECCHIA, D. L. 1981. Effects of streamflow and upwelling on yield of wild coho salmon (*Oncorhynchus kisutch*) in Oregon. Can. J. Fish. Aquat. Sci. 38: 471–475.

SMITH, B. D., G. A. MCFARLANE, AND M. W. SAUNDERS. 1980. Variation in Pacific hake (*Merluccius productus*) summer length-at-age near southern Vancouver Island and its relationship to fishing and oceanography. Can. J. Fish. Aquat. Sci. 47 (In press).

STOCKER, M., V. HAIST, AND D. FOURNIER. 1983. Stock assessments for British Columbia herring in 1982 and forecasts of the potential catch in 1983. Can. Tech. Rep. Fish. Aquat. Sci. 1158: 53 p.

THOMSON, R. E., B. M. HICKEY, AND P. H. LEBLOND. 1989. The Vancouver Island coastal current: fisheries barrier and conduit, p. 265–296. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. Can. Spec. Publ. Fish. Aquat. Sci. 108.

WALTERS, C. J., R. HILBORN, R. M. PETERMAN, AND M. J. STALEY. 1978. Model for examining early ocean limitation of Pacific salmon production. J. Fish. Res. Baord Can. 35: 1303–1315.

WALTERS, C. J., M. STOCKER, A. V. TYLER, AND S. J. WESTRHEIM. 1986. Interaction between Pacific cod (*Gadus macrocephalus*) and herring (*Clupea harengus pallasi*) in the Hecate Strait, British Columbia. Can. J. Fish. Aquat. Sci. 43: 830–837.

WARE, D. M., AND G. A. MCFARLANE. 1988. Relative impact of Pacific hake, sablefish and Pacific cod on west coast of Vancouver Island herring stocks. Int. N. Pac. Fish. Comm. Bull. 47: 67–77.

Variation in Pacific Hake (*Merluccius productus*) Summer Length-at-Age Near Southern Vancouver Island and Its Relationship to Fishing and Oceanography

Barry D. Smith, Gordon A. McFarlane, and Mark W. Saunders

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, British Columbia V9R 5K6 Canada

Smith, B. D., G. A. McFarlane, and M. W. Saunders. 1990. Variation in Pacific hake (*Merluccius productus*) summer length-at-age near southern Vancouver Island and its relationship to fishing and oceanography. Can. J. Fish. Aquat. Sci. 47: 2195–2211.

Male and female Pacific hake (*Merluccius productus*) sampled in summer off southern Vancouver Island since 1976 show a general decline, plus marked variability, in mean lengths-at-age. Multiple regression analysis related the length-at-age pattern to von Bertalanffy and density-dependent growth, length-selective fishing mortalities, and a suite of oceanographic covariates (sea level height, temperature, and salinity anomalies). Because offshore Pacific hake migrate annually between southern California and British Columbia, with larger fish migrating further north, we deduce that the summer fishery in Canadian waters selectively removes the larger hake each year causing a decline over time in mean lengths-at-age. Apart from the general decline, oceanographic factors explained most interannual variability in mean lengths-at-age. Specifically, mean lengths-at-age were generally smaller in years of above normal sea level height and temperature, and below normal salinity. Sea level anomalies in particular have been associated with the relative strength of the alongshore currents within the coastal upwelling domain; thus, we argue that stronger northward (or weaker southward) flows assist the northward migration of hake. Consequently, in years of higher than normal sea level, such as during major El Niño–Southern Oscillation (ENSO) events, more smaller hake arrive off Vancouver Island in summer resulting in lower mean lengths-at-age.

Les pêches de merlu du Pacifique mâles et femelles (*Merluccius productus*) échantillonnées pendant l'été au large de la côte sud de l'île de Vancouver affichent depuis 1976 une diminution générale et une variabilité marquée des longueurs moyennes en fonction de l'âge. L'analyse de régression multiple reliait le modèle de longueur en fonction de l'âge à la croissance von Bertalanffy et à la croissance en fonction de la densité, à la mortalité des poissons par longueur et à une suite de covariables océanographiques (anomalies du niveau de la mer, de température et de salinité). Comme le merlu du Pacifique au large des côtes entreprend annuellement une migration qui le conduit des côtes sud de la Californie jusqu'à la Colombie-Britannique et que les plus gros merlus vont encore plus au nord, on conclut que la pêche estivale dans les eaux canadiennes entraîne un prélèvement sélectif des plus gros merlus chaque année, ce qui cause avec le temps une réduction des longueurs moyennes en fonction de l'âge. Outre la diminution générale, des facteurs océanographiques expliquent la majeure partie de la variabilité des longueurs moyennes en fonction de l'âge d'une année à l'autre. Plus précisément, les longueurs moyennes/âge étaient en général plus faibles les années où l'on observait une température et un niveau de la mer supérieurs à la normale ainsi qu'une salinité inférieure à la normale. Des anomalies dans la hauteur du niveau de la mer, en particulier, ont été associées à la force relative des courants littoraux dans la zone côtière à marée. On conclut donc que les courants plus forts vers le nord (ou plus faibles vers le sud) facilitent la migration du merlu vers le nord. Par conséquent, les années où le niveau de la mer est plus élevé que la normale, par exemple lors d'événements causés par l'oscillation méridionale El Niño, un plus grand nombre de merlus de plus petite taille arrivent au large de l'île de Vancouver pendant l'été, ce qui explique que les longueurs moyennes/âge soient inférieures.

Received February 8, 1990
Accepted May 10, 1990
(JA463)

Reçu le 8 février 1990
Accepté le 10 mai 1990

Pacific hake (*Merluccius productus*) inhabit the coastal waters off the west coast of North America (Fig. 1) at depths of 100–250 m (Stauffer 1985). Most Pacific hake belong to an offshore migratory population which exends from southern California to British Columbia (Stauffer 1985), but there are also distinct populations of Pacific hake in the Strait of Georgia (McFarlane and Beamish 1985), Puget Sound (Pedersen 1985) and inlets along the west coast of Vancouver Island (Beamish and McFarlane 1985; Shaw et al. 1985, 1989). All of these populations are commercially exploited except the several small and somewhat isolated inlet populations. Of greatest importance is the offshore population because of its large yield for fisheries in both Canadian and American coastal waters (Saunders and Shaw 1989). This population attracts particular scientific attention because only the largest hake migrate north to Canadian waters (Beamish and McFarlane 1985) and dramatic variability in year-class strength (Bailey and Francis 1985) affects the interannual distribution of biomass, and consequently yield, of hake between Canada and the United States (Swartzman et al. 1987).

Offshore Pacific hake are believed to spawn between January and March each year at mid-depths (100–500 m) within a few hundred kilometres off the coast of southern California or northern Mexico (Bailey et al. 1982, Bailey and Francis 1985).

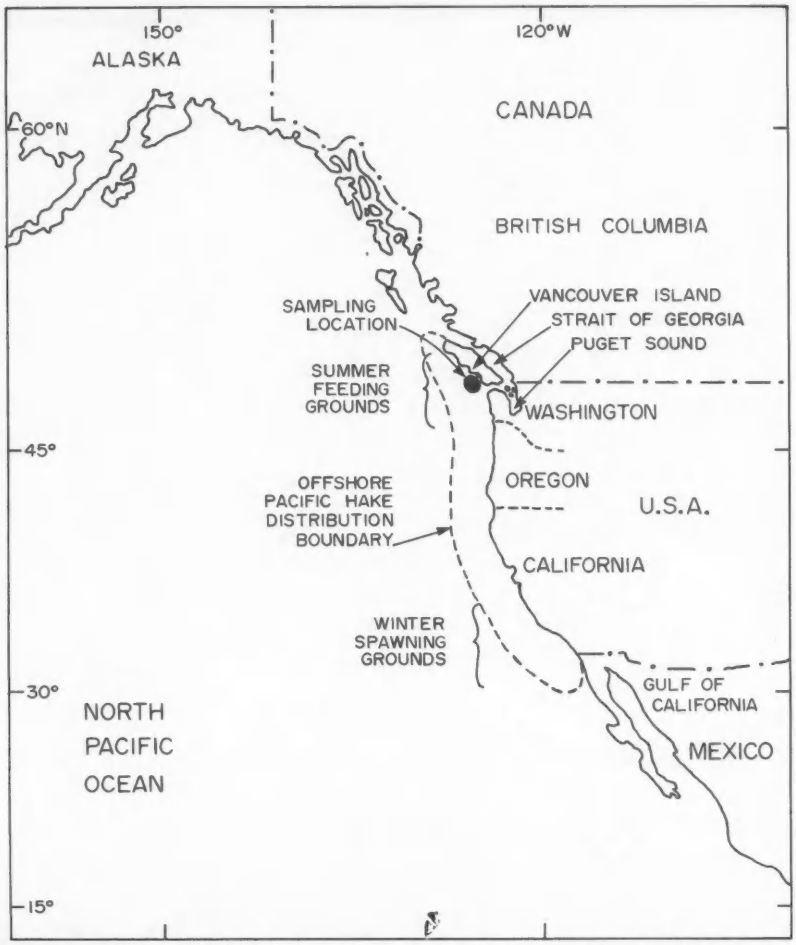

FIG. 1. Map of the west coast of North America indicating the location of distinct Pacific hake populations offshore, in the Strait of Georgia, and in Puget Sound. The samples analyzed for this paper were all collected within the indicated sampling area between June and October each year from 1976 until 1988 except 1977.

Small, young hake (up to about 2 yr old) live year-round shoreward of where they were spawned (Bailey and Francis 1985; Beamish and McFarlane 1985). As they grow, they begin an annual feeding migration, the northern extent of which increases with fish age (Beamish and McFarlane 1985), but which is most likely related to fish length (Francis 1983) and optimum swimming speed (Ware 1978; Weihs and Webb 1983). The northern extent of the annual migration of the largest fish has not been precisely determined, but for each year sampled, the largest summer concentrations of offshore hake in Canadian waters occur near southern Vancouver Island (Beamish 1981; Beamish et al. 1982; Barner et al. 1984; Shaw et al. 1987, 1989) where oceanographic conditions favour high productivity (Thomson and Ware 1988; Ware and McFarlane 1989).

Fishable concentrations of hake occur in Canadian waters from about June until October of each year (Beamish 1981; Beamish and McFarlane 1985). Since 1968 Pacific hake landed to either domestic onshore or joint venture offshore processors often produced the largest catch, by weight, of any Canadian west coast groundfish (Saunders and Shaw 1989). Pacific hake are usually 5 yr old when they begin to occur in abundance in Canadian waters during summer, but summer samples frequently contain some fish as young as 3 yr old. By November, hake abundance in Canadian waters has sharply declined due to the southern migration for spawning which follows the transition to winter oceanographic conditions (Thomson and Ware 1988). From a recent low of about 3.4 kt in 1977, Pacific hake landings from Canadian waters have steadily increased with the 90.5 kt landed in 1988 being second only to the 93.9 kt landed in 1969 (Saunders and Shaw 1989).

Conceptually, differential fishing mortality upon a population length-segregated by migration, and variable oceanographic conditions operating in conjunction with length-dependent migration rates, can markedly modify the expected transboundary distribution of offshore Pacific hake biomass off southern Vancouver Island during summer. Underestimating the importance of oceanography and selective fishing would have immediate implications in terms of inaccurate short-term

forecasts of biomass distribution in any particular year, but perhaps more importantly, a misunderstanding of the fishery and oceanographic factors might lead to improper long-term management with serious implications for the offshore hake stock as well as the transboundary availability of fishable concentrations of Pacific hake.

These concerns led us to investigate the interannual variability in Pacific hake mean lengths-at-age apparent in our summer samples collected since 1976 for clues about the oceanographic and fishery processes affecting the transboundary distribution of hake. Our anticipation of some enlightenment on the effects of such processes was bolstered both by the data supporting Bailey's (1980) hypothesis that selective removal of large adults from the mid-1960's until 1979 increased the mean latitude where spawning occurred, i.e. a shorter migration route; and by Mysak's (1986) proposal that anomalously strong northward flows of coastal currents during major El Niño-Southern Oscillation (ENSO) events might assist the northern migration of young (i.e. small) hake. Mysak (1986) also proposed that warm water further north than usual, such as during major ENSO events, might result in spawning occurring closer to the summer feeding grounds. Such phenomena could have important consequences for hake population dynamics. For example, reaching the productive waters off southern Vancouver Island early might increase growth and survival rates.

We identified the following list of processes which could hypothetically introduce interannual variability to the length-at-age composition of our offshore Pacific hake samples.

1) Systematic ageing and/or measuring errors.
2) Interannual variability in the degree of mixing of different Pacific hake populations, e.g. offshore, Strait of Georgia, Puget Sound, west coast inlets.
3) Growth rate variability related to food availability, population density, and/or temperature.
4) Length-selective fishing and/or natural mortality over time.
5) Length-dependent migration rates operating in conjunction with:
   a) interannual variability in the location of spawning, and thus the migration distance to the feeding grounds off Vancouver Island; and
   b) oceanographic factors affecting the route and rate of the annual migration.

   (Both processes could affect the transboundary

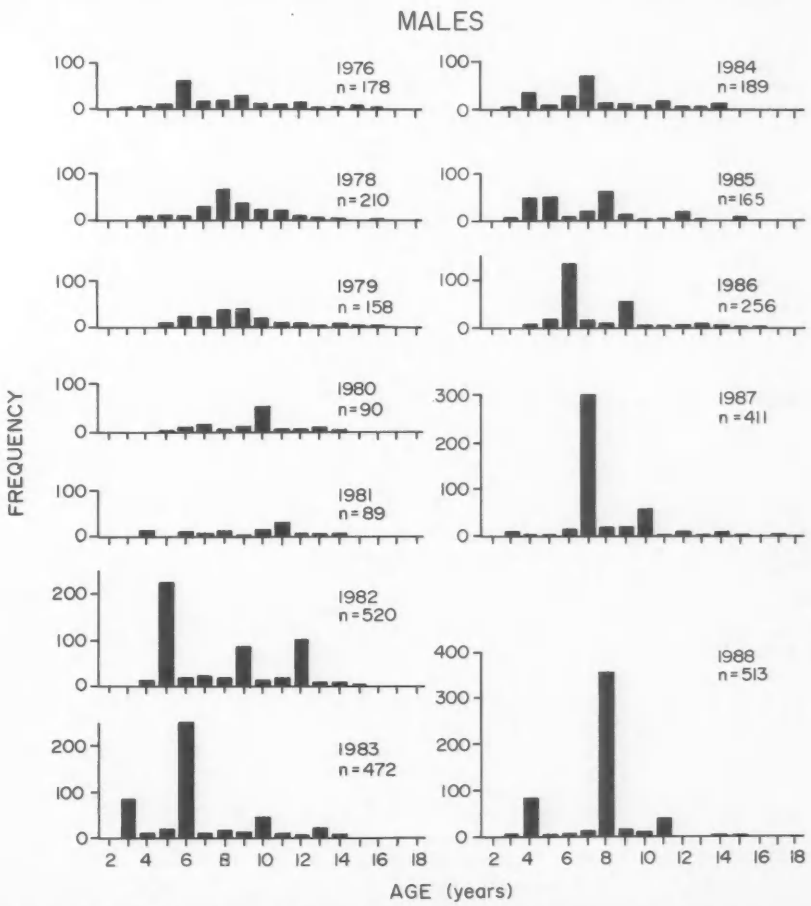

FIG. 2. Age distributions for male offshore Pacific hake collected from the shelf area off Vancouver Island between 48 °20′N and 49°N from 1976 until 1988.

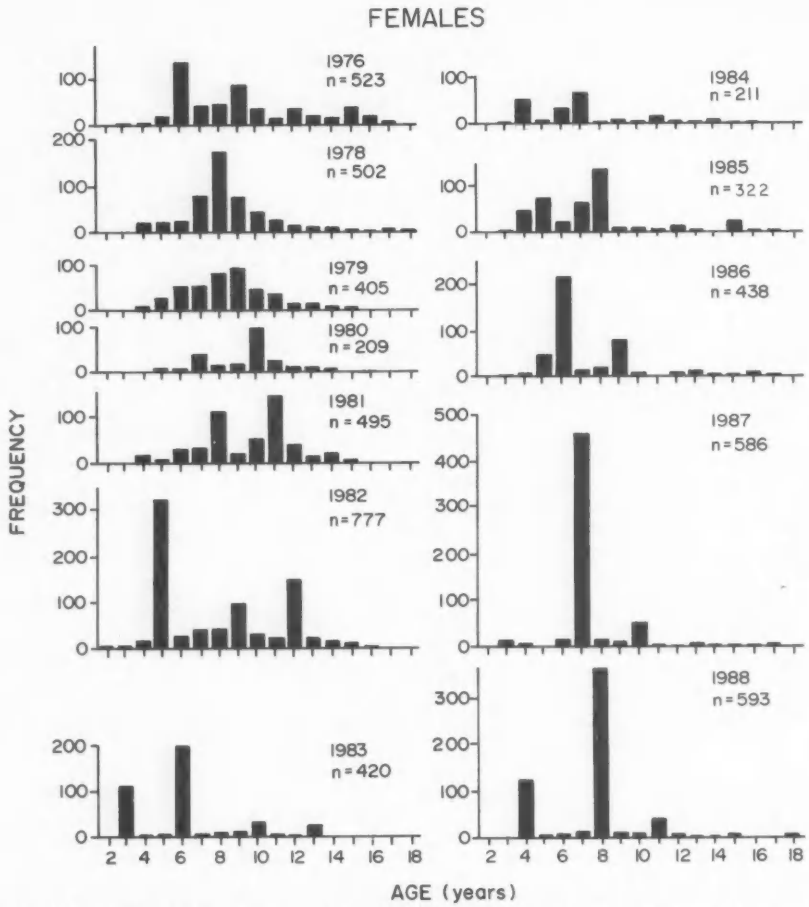

FIG. 3. Age distributions for female offshore Pacific hake collected from the shelf area off Vancouver Island between 48°20′N and 49°N from 1976 until 1988.

length and length-at-age distributions of Pacific hake thereby invoking length-selective fishing and sampling bias.)

6) Interannual variability in sampling bias related to the age and length structure of the Pacific hake schools preferentially exploited by the fishery.

Of these processes, age determination from otolith annuli by the break and burn technique of Beamish (1979) has proven very reliable when performed by experienced technicians. Validation of this method has essentially been achieved by having consistently observed a unit increase in age each year for those dominant year-classes sampled over time (McFarlane and Beamish 1985). Pacific hake can be consistently measured for fork length to the nearest few millimetres, therefore, we do not consider ageing or measuring errors to be plausible sources of the systematic interannual variation in mean lengths-at-age of the magnitude we observed.

Interannual variation in the degree of mixing of offshore, Strait of Georgia, and Puget Sound hake stocks on the feeding grounds off southern Vancouver Island is also an unlikely source of interannual variation in mean lengths-at-age, despite Strait of Georgia hake being smaller in length than offshore hake (Beamish and McFarlane 1985; Welch and McFarlane 1990). Beamish and McFarlane (1985) refer to the asynchrony of dominant year-classes, different otolith morphologies, and the absence of the myxosporean parasite *Kudoa paniformis* (Kabata and Whitaker 1981, 1986), to argue that Strait of Georgia hake form a population discrete from offshore hake. Further, summer sampling in Juan de Fuca Strait, a transition area between the two populations, yielded hake whose year-class composition was typical of offshore hake and not Strait of Georgia hake (Beamish et al. 1982). Similarly, one would not expect many Puget Sound hake to mix with offshore hake off southern Vancouver Island.

We are more cautious about Pacific hake from British Columbia west coast inlet populations (Beamish and McFarlane 1985; Shaw et al. 1985, 1989) mixing with offshore Pacific hake as they mature, thereby contaminating length-at-age samples for offshore hake. The interannual pattern in mean lengths-at-age of 3- and 4-yr-old male and female hake sampled in summer off southern Vancouver Island was more variable than that for the older hake, and did not appear to display a declining trend. We suspect many of these 3- and 4-yr-old hake might have been spawned locally since, as mentioned previously, few

TABLE 1. Sea level height (Tofino, British Columbia), surface temperature and salinity (Amphitrite Point, B.C.) means and related statistics.

	Sea level height (cm)	Surface temperature (°C)	Surface salinity (‰)
Year series started	1910	1935	1935
Number of complete years[a]	53	49	53
Overall mean value[b]	214.852	10.374	29.540
Overall SD	12.574	2.154	1.284
Minimum monthly mean	172.300	5.170	25.200
Maximum monthly mean	271.580	14.900	32.350

[a]Years for which all 12 monthly mean values were available.
[b]Based on years with all 12 monthly mean values available.

offshore hake younger than 5 yr migrate as far north as Canada (Beamish and McFarlane 1985). Hake older than about 6 yr have rarely been taken in inlet samples so it is possible that as hake in coastal inlets mature they eventually mix with the offshore population; however, the number of older hake from inlet populations would be a low proportion of the offshore population. Consequently, the analysis we present in this paper does not incorporate data for hake younger than 5 yr and we dismiss interannual variability in the degee of mixture of hake populations as a plausible explanation for interannual variability in offshore Pacific hake mean lengths-at-age.

The elimination of processes 1 and 2 in the above list permitted us to focus on the remaining more plausible hypotheses (processes 3–6) as explanations for interannual variation in Pacific hake mean lengths-at-age. Among the remaining hypotheses there is a logical sequence in which processes 3–6 would introduce variability to our length-at-age samples. Firstly, *variability in mean length-at-age due to growth* could be generated by variability in the rate of growth of individual fish (i.e. process 3). Secondly, lengths-at-age predicted from growth alone could be modified by length-selective mortalities, i.e. process 4 (Lee 1912; Ricker 1969), generating *population mean lengths-at-age* that misrepresent real growth rates. Thirdly, *sample mean lengths-at-age* estimated from our hake collections could misrepresent population lengths-at-age because of the effects of length-dependent migration rates, oceanographic factors affecting migration rates, fish schooling behavior, and the selective targeting of schools by the fishery (processes 5 and 6). To address these possibilities we incorporated covariate time series of estimated population biomass; the annual percent (by weight) of the estimated population biomass of Pacific hake landed from Canadian waters (Saunders and Shaw 1989); and local sea level height, temperature, and salinity anomalies (Dodimead 1984) into our analysis. Each of these population, fishery, and oceanographic time series proved to bear a significant and plausible statistical relation to the dynamic processes 3 to 6.

Sampling

Mean lengths-at-age of offshore Pacific hake were determined from random samples of Pacific hake taken from midwater trawl catches. Samples were obtained either by technicians on board research vessels operated or chartered by the Pacific Biological Station, or by "observers" on foreign commercial vessels (Davenport 1985). Offshore Pacific hake have been collected routinely between late June and mid-October since 1976, with 1977 being the only year when no samples were collected. Samples have been obtained from as far south as Oregon to north of Vancouver Island, but in this paper we analyse only those samples collected from the shelf area off southern Vancouver Island between 48°20′N and 49°N (Fig. 1). Individual hake were sampled to determine their sex and fork length to the nearest millimetre. The largest pair of otoliths was collected for age determination according to the procedures of Beamish (1979). There were generally few hake 14 yr and older in our samples (Fig. 2, 3), and they showed little interage variation in length, so for the analysis presented herein we combined all hake 14 yr and older into a 14$^{>}$ age-class with a larger sample size.

Oceanographic Data

We chose time series of mean monthly values for sea level height (unadjusted for local atmospheric pressure variation), sea surface temperature, and sea surface salinity obtained from shore stations at Amphitrite Point (temperature and salinity) and Tofino (sea level height), British Columbia as gross indicators of coastal oceanographic variability from California to British Columbia. We used only our southern British Columbia data because a few recent studies have documented considerable similarity among comparable time series from stations along the migration route of offshore Pacific hake (Chelton and Davis 1982; Emery and Hamilton 1985; Mysak 1986). Further, data from British Columbia stations have been shown to be indicative of large scale oceanographic events (e.g. ENSO events) propagated from further south (Emery and Hamilton 1985; Mysak 1986).

These conclusions stem from most of the northeast Pacific being under the influence of two dominant climatological pressure regimes: the Aleutian low pressure system during winter and the North Pacific high pressure system during summer (Favorite et al. 1976; Thomson 1981; Dodimead 1984; Emery and Hamilton 1985). Winds associated with these two pressure systems drive the dominant oceanic currents such as the equatorward flowing surface California Current (up to 850–900 km offshore), and the poleward flowing surface Alaska Current (Tabata 1975). They also generate the seasonal pattern of coastal surface currents and onshore and offshore Ekman transport (Dodimead 1984; Freeland et al. 1984; Thomson et al. 1989).

In winter, the coastal and generally northward flowing Davidson Current (off California, sometimes referred to as the California Countercurrent, e.g. McLain and Thomas (1983)) is responsible for most of the nearshore and alongshore movement of water from California to British Columbia. After the spring transition (Thomson and Ware 1988), strong northwest winds deflect surface water offshore (Ekman transport) generating a marked equatorward flowing surface current over the continental margin from northern Vancouver Island to California. Subsurface flow (deeper than 300 m) continues poleward as the California Undercurrent (Hickey 1979; McLain and Thomas 1983; Lynn and Simpson 1987; Thomson et al. 1989). Offshore Pacific hake reside over the continental shelf at 100–250 m depth (Stauffer 1985) and are found mainly in the domain of the coastal surface currents. Thus it would seem that the relative flow rates of these currents could affect hake migration rates. Field studies have shown that both intra-annual and interannual variability in the strength of coastal surface currents are rec-

TABLE 2. Definitions and units for symbols used to represent model variables and parameters. Asterisks in the Symbol column indicate the nine explicitly estimated parameters.

Symbol	Units	Definition
$O_{i,y}$	cm	Observed mean length-at-age i in year y
$L_{i,y}$	cm	Mean length-at-age due to growth alone
$P_{i,y}$	cm	Population mean length-at-age
$S_{i,y}$	cm	Predicted mean length-at-age of samples
$\sigma_{i,y}$	cm	Standard error of $O_{i,y}$
$L_{5,1976}$*	cm	Mean length-at-age 5 in year 1976
i	yr	Index for ages $i=1,\ldots,14^{>}$ yr
y	yr	Index for years $y=1963,\ldots,1988$
L_∞*	cm	Asymptotic length achieved by growth
K*	yr^{-1}	Instantaneous rate of change in growth rate
T_0	yr	Age when fish length extrapolates to 0 cm
δ*	kt^{-1}	Coefficient for density-dependence on B_y
C_y	kt	Tonnage of offshore Pacific hake landed from Canadian waters in year y
B_y	kt	Estimated population biomass of offshore hake age 2 and older in year y
ϵ*	cm	Coefficient of length-selective fishing mortality related to C_y/B_y
λ*	cm	Coefficient of length-selective fishing and natural mortality related to B_y
β_1*	$cm \cdot cm^{-1}$	Coefficient relating sea level height anomalies to changes in lengths-at-age
Ω_y	cm	Cumulative sea level height anomalies for 12 mo prior to August in year y
β_2*	$cm \cdot °C^{-1}$	Coefficient relating surface temperature anomalies to changes in lengths-at-age
Θ_y	°C	Cumulative surface temperature anomalies for 12 mo prior to August in year y
β_3*	$cm \cdot ‰^{-1}$	Coefficient relating surface salinity anomalies to changes in lengths-at-age
Φ_y	‰	Cumulative surface salinity anomalies for 12 mo prior to August in year y
f	dimensionless	Negative log-likelihood

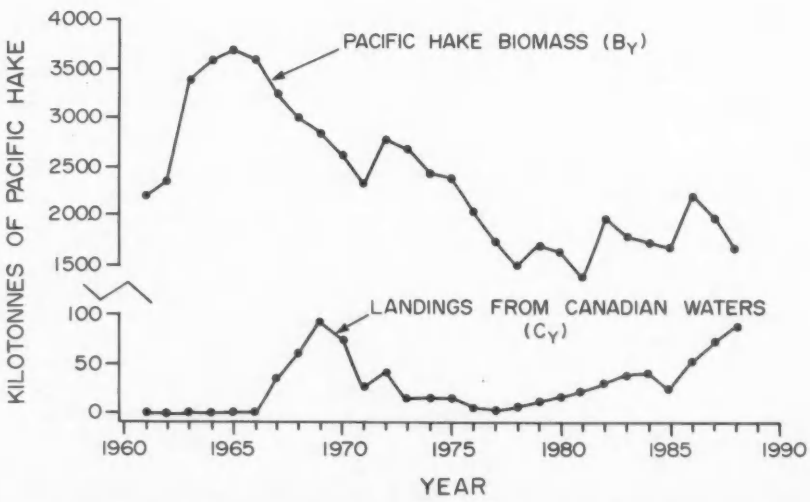

FIG. 4. Mean annual estimated population biomass of offshore Pacific hake age 2 and older (M. Dorn and R. Methot, unpubl. data) and the annual tonnage of offshore Pacific hake landed from Canadian waters.

ognizable in oceanographic time series, particularly sea level height (Chelton and Davis 1982; Emery and Hamilton 1985).

We converted values of sea level height, surface temperature, and surface salinity to anomalies of the long-term mean values. Means were calculated using only the data for those years in which all 12 monthly means were available in order to eliminate a seasonal bias (Table 1). For our analysis we required only that segment of the time series beginning in 1962. A few missing monthly mean values for sea level height (due to failure of the recording device) were estimated using data from a sea level height recorder at nearby Bamfield, British Columbia, which were corrected for site differences.

Model Development

We used a non-linear multiple regression analysis to estimate the parameters which minimized the discrepancy between predicted and observed values of mean lengths-at-age for ages 5–14$^{>}$ in years 1976, and 1978–1988. We summarize the variables used in our analysis in Table 2. Following the logic developed in our introduction, we first assumed von Bertalanffy growth for estimating the mean lengths-at-age for Pacific hake resulting from growth alone. Francis (1983) found von Bertalanffy growth satisfactory for offshore Pacific hake as did Welch and McFarlane (1990) for female Strait of Georgia Pacific hake. The von Bertalanffy growth function,

$$(1)\quad L_{i,y} = P_{i-1,y-1} + (L_\infty - P_{i-1,y-1})\,(1 - e^{-k}),$$

was entered into our explanatory model as a difference equation with length-at-age i in year y ($L_{i,y}$, $i=1,\ldots,14^{>}$, $y=1963,\ldots,1988$) as a function of $P_{i-1,y-1}$. The variable $P_{i-1,y-1}$ represents population mean length-at-age $i-1$ in year $y-1$, L_∞ is asymptotic length and K is the annual instantaneous rate of change in growth rate toward L_∞. This formulation allowed us to include annual density-dependent growth yielding

$$(2)\quad L_{i,y} = P_{i-1,y-1} + (L_\infty - P_{i-1,y-1})\,(1 - e^{-k})e^{\delta(B_y - B_{1976})}$$

where the B_y's (Fig. 4) are estimates of mean annual biomass for offshore Pacific hake age 2 and older (M. Dorn and R. Methot, National Marine Fisheries Service, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle WA, unpubl. data), and δ is the coefficient of density-dependence. We standardized our growth parameter estimates to B_{1976} because B_{1976} was a typical population biomass estimate and the fishery in Canadian waters was small in 1976 (Fig. 4).

Growth variability due to density-dependent factors is potentially greatest during early life when growth rates are fastest, therefore we began our hake growth model during 1963 when the oldest cohort in our data set ($14^{>}$yr) was 1^{+} yr old. We assumed there was no density-dependent growth before age 1 for all cohorts contributing to our data set, thus

$$(3)\quad L_{i,y} = L_{5,1976} + (L_\infty - L_{5,1976})\,(1 - e^{-k(i-5)})$$

for $i=1$ and $y=1963,\ldots,1984$. Our data set contains information only for hake 5 yr and older so we chose length-at-age 5 in 1976 ($L_{5,1976}$), instead of the more traditional von Bertalanffy variable T_o, to scale length to age (Schnute 1981).

We introduced two length-selective mortality processes which we believed could produce the population lengths-at-age ($P_{i,y}$) upon which Eq. 1 and 2 depend. We relate the first process of a general length-selective total mortality to B_y under the assumption that lower B_y's indicate recent population histories

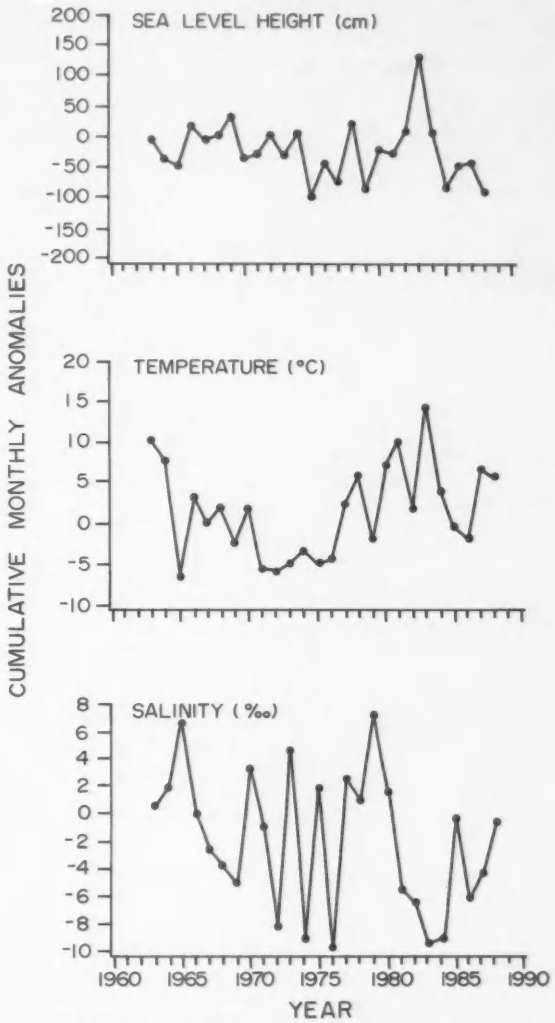

FIG. 5. Cumulative mean monthly anomalies of sea level height (Ω_y), temperature surface (Θ_y) and surface salinity (Φ_y) for the 12 mo from August of year y-1 until July of year y beginning year $y=1963$.

influenced more by mortality (both natural and fishing) than recruitment, and vice versa. By using Eq. (4) we essentially accept Lee's (1912) proposal that faster growing fish have a higher mortality rate than slower growing fish resulting in population lengths-at-age less than those predicted by back-calculation from growth increments on scales or otoliths, i.e. "Lee's pheomenon."

If the rate of length-selective instantaneous mortality is assumed proportional to relative population biomass (expressed instantaneous) then $P_{i,y}$ can be related to $L_{i,y}$ by

$$(4)\quad P_{i,y} = L_{i,y} + \lambda \log_e (B_y/B_{1976})$$

where λ is the regression coefficient and, as for Eq. 2, 1976 is the reference year. Equation 4 is justified by Jones' (1958; see also Ricker (1969)) demonstration that an instantaneous length-selective mortality rate will change the mean length-at-age i for

a normally distributed age-class by that rate times the variance in length-at-age i. Thus if we assume equal variances for age-classes 5–14>, then $\lambda \log_e(B_y/B_{1976})$ divided by the variance in length-at-age would estimate the instantaneous rate of length-selective mortality.

When we add an additional length-selective process related to the annual proportion of the hake population biomass taken by the fishery in Canadian waters (C_y/B_y), Eq. 4 is extended to

$$(5)\quad P_{i,y} = L_{i,y} + \lambda \log_e(B_y/B_{1976}) + \epsilon(C_y/B_y)$$

where ϵ is the regression coefficient associated with C_y/B_y. This additional process affecting population mean length-at-age is based on the assumption that length-dependent migration rates result in only the largest hake reaching Canadian waters, and that these hake are selectively removed by the fishery in Canadian waters. We chose to make $P_{i,y}$ dependent upon C_y/B_y rather than C_{y-1}/B_{y-1} because preliminary analyses determined that $P_{i,y}$ bore closer statistical relationship to C_y/B_y than C_{y-1}/B_{y-1}. This possibly indicates that, in general, population lengths-at-age ($P_{i,y}$) at the time of sampling in year y have already been modified by the fishery in year y.

Lastly, we assumed the mean lengths-at-age of our offshore Pacific hake samples ($S_{i,y}$) did not accurately represent population mean lengths-at-age ($P_{i,y}$). We attribute the discrepancy between $S_{i,y}$ and $P_{i,y}$ to length-segregation of the offshore hake population in summer due to length-dependent migration rates and interannual variability in oceanographic conditions affecting migration rates. Essentially we presumed that (1) interannual variation in the relative flow rates of the coastal surface currents affects the length distribution of hake reaching Canadian waters, (2) our oceanographic data are related to this variability, and (3) our samples reflect this variability.

Regression coefficients for sea level height (β_1), surface temperature (β_2), and surface salinity (β_3) anomalies entered the explanatory model linearly as

$$(6)\quad S_{i,y} = P_{i,y} + \beta_1\Omega_y + \beta_2\Theta_y + \beta_3\Phi_y$$

where $S_{i,y}$ represents the expected length-at-age composition of hake sampled from Canadian waters. The variables Ω_y (sea level height), Θ_y (surface temperature), and Φ_y (surface salinity) represent cumulative monthly anomalies for the 12 mo from August of year $y-1$ until July of year y (Fig. 5) because early August was the mean collection time for our offshore Pacific hake samples. By using cumulative anomalies and choosing a lag of 12 mo, we presume that the relative flow rate of the coastal surface currents during the entire year prior to sampling is a determinant of the geographical distribution of hake by length, and therefore also of the expected length-at-age composition of our samples. In trial analysis we improved our model fit modestly by estimating the lags as well as the coefficients β_1, β_2 and β_3. However, the results were inconsistent between the analyses of the male and female data sets and there was no obvious biological or oceanographic explanation for the alternate lags.

Our model was fit to our observed mean lengths-at-age ($O_{i,y}$) separately for male and female hake assuming a normal distribution of errors. We used the negative log-likelihood function

$$(7)\quad f = 0.5 \sum_{x,y} ((S_{i,y} - O_{i,y})/\sigma_{i,y})^2 i,y),$$

which is a weighted sum of squares, to minimize the discrepancy between $S_{i,y}$ and $O_{i,y}$. Equation 7 includes the standard errors ($\sigma_{i,y}$) for the observed mean lengths-at-age $O_{i,y}$ (Zar 1984), thereby giving more weight to those mean lengths-at-age better sampled. Values for $O_{i,y}$ based on only one fish were excluded from the calculation of f, because $\sigma_{i,y}$ is undefined for a sample size of one, leaving 115 $O_{i,y}$ for both males and females. In total, nine parameters required simultaneous estimation: $L_{5,1976}$, L_∞, K, δ, ϵ, λ, β_1, β_2, and β_3.

Our model structure was non-linear because of the von Bertalanffy growth assumption, therefore we used the SIMPLEX algorithm of Nelder and Mead (1965) as implemented by Mittertreiner and Schnute (1985) to minimize the likelihood function f, at which point $f_{min} = f$. Confidence intervals for the

TABLE 3. Analysis of variance for those processes proposed to describe the length-at-age pattern of male and female offshore Pacific hake sampled off southern Vancouver Island between July and October of 1976–88. The sum of squares (SS) for any process is the sum of the squared residuals $(S_{i,y} - O_{i,y})^2$ obtained by minimizing Eq. 7 with that process excluded, minus the residual error SS for all processes included. The corresponding r^2 for any process is thus the proportion of the total SS added to residual error SS by excluding that process from the model.

Source of variance	df	SS	Mean SS	r^2	$F_{(df,106)}$	p	f_{min}
			Male				
Process							
von Bertalanffy growth	3	697.8	232.6	0.733	118.7	<0.001	1196.4
Density-dependent growth	1	100.0	100.0	0.105	51.0	<0.001	205.2
General length-selectivity	1	115.0	115.0	0.121	58.7	<0.001	201.0
Selective Canadian fishery	1	106.3	106.3	0.112	54.2	<0.001	347.8
Oceanography	3	94.3	31.4	0.099	16.0	<0.001	247.5
Residual error	106	207.8	1.96	0.218			164.5
Total	114	952.4					
			Female				
Process							
von Bertalanffy growth	3	1400.1	466.7	0.949	700.1	<0.001	1787.3
Density-dependent growth	1	37.8	37.8	0.026	18.9	<0.001	219.9
General length-selectivity	1	58.4	58.4	0.040	29.2	<0.001	230.3
Selective Canadian fishery	1	172.5	172.5	0.117	86.3	<0.001	360.5
Oceanography	3	107.7	35.9	0.073	18.0	<0.001	313.9
Residual error	106	211.8	2.00	0.143			168.0
Total	114	1476.1					

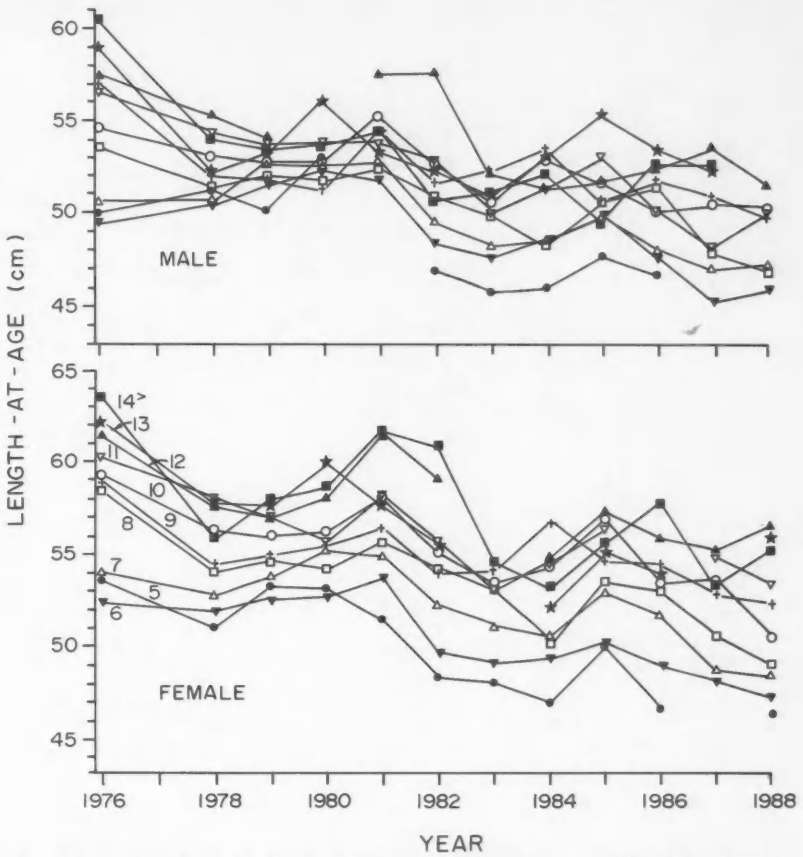

FIG. 6. Time series of mean lengths-at-age for ages 5–14>, where age-class 14> includes all offshore Pacific hake 14-yr and older. Note four features in the length-at-age pattern: (1) an annual increase in mean lengths-at-age with increasing age, (2) a general decline in mean lengths-at-age with time, (3) interannual variation in mean lengths-at-age in addition to (2), and (4) interannual variation in the range of mean lengths-at-age for each year.

parameter estimates were calculated directly from Eq. 7 using the approximate numerical method supplied with Mittertreiner and Schnute's (1985) SIMPLEX package. This method uses the matrix of second partial derivatives of the likelihood function with respect to the parameters (calculated numerically) to generate the asymptotic covariance matrix (Kendall and Stuart 1979).

Model Results and Credibility

Statistics

Analysis of variance (Table 3) indicated that all 5 modelled processes significantly ($p<0.001$, $r^2=0.78$ and 0.86 for males and females, respectively) reduced the residual sum of squares $(S_{i,y}-O_{i,y})^2$ for our observed length-at-age pattern (Fig. 6). The r^2 values for each modelled process indicate that growth was the most important determinant of mean length-at-age variability, followed by length-selectivity by the fishery in Canadian waters. The r^2 values for processes other than growth are considerably less than those for growth, although for any single age-class the r^2 values for the remaining three processes account for all explained variability. The r^2 values for the effect of oceanographic factors on the length-at-age variability of our samples are only 0.099 and 0.073 for males and females, respectively, yet a comparison of Fig. 7 (predicted lengths-at-age with all processes included) with Fig. 8 (predicted lengths-at-age assuming no oceanographic influences on hake length-at-age distribution in Canadian waters) demonstrates the importance of oceanographic factors in generating a predicted length-at-age pattern which captures the interannual variability in the general decline in observed lengths-at-age (Fig. 6).

The overlap of 95% C.I. for most equivalent parameter estimates in the male and female analyses indicate that the modelled processes affect males and females similarly (Table 4). The typical exception was that female hake were larger at a given age than male hake with both $L_{5,1976}$ and L_∞ being significantly larger for females. Acceptable levels of correlations between all parameter pairs in both the male and female analyses (Table 5), and near normal distributions of residuals indicate that our model bears a sound statistical relationship to the data. There appears to remain a weak

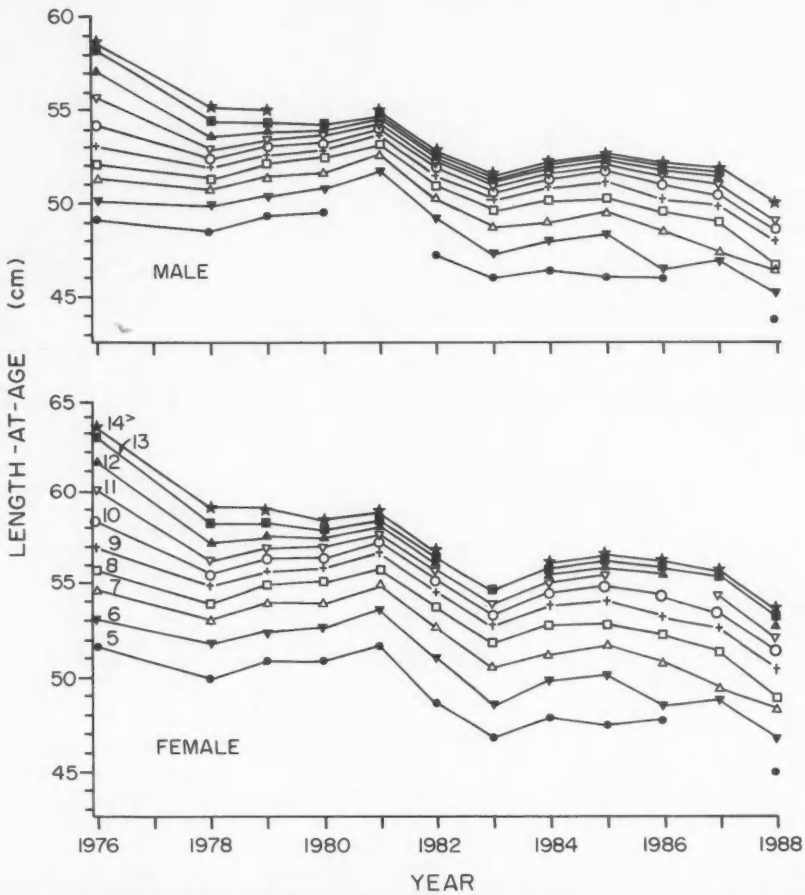

FIG. 7. Predicted pattern of mean lengths-at-age 5–14> using the maximum likelihood parameter estimates for all five processes proposed to describe the length-at-age pattern of male and female offshore Pacific hake sampled off southern Vancouver Island between July and October of 1976 until 1988.

interannual pattern in the standardized female residuals vs. year of sampling (Fig. 9), but we were unable to remove this pattern with any simple and justifiable modifications to the described model.

We remain suspicious that interannual variability in sampling bias related to the age and length structure of the Pacific hake schools preferentially exploited by the fishery (process 6 in the Introduction) might be contributing to the residual pattern in Fig. 9, but we had no success relating this pattern to our most relevant data: interannual variability in hake age distribution (Fig. 2, 3). Conceivably, dominant age-classes (and consequently dominant length-classes) within the hake population could be the focus of a fishery such that the length composition of adjacent age-classes within the catch could be biased toward that of the dominant age-class. Note that in the years 1978 until 1980 the ranges in mean lengths-at-age were markedly less than predicted for both males and females which to us seems most likely explained by us having sampled hake schools structured by length, not age.

Growth

The three von Bertalanffy growth parameter estimates, and the parameter δ measuring the instantaneous rate of change in growth rate due to dependence on hake population biomass, suggest a plausible range of growth variability over the range of B_y's observed (Fig. 10). Note that the growth functions for the extreme B_y's of $B_{1965} = 3695$ kt (maximum B_y) and $B_{1981} = 1384$ kt (minimum B_y) would never be observed over an extended period in nature because of large interannual variability in population biomass. Predicted mean lengths-at-age 1 near 30–35 cm agree well with previous estimates for offshore Pacific hake this age (Francis 1983; Beamish and McFarlane 1985), especially when it is considered that hake of age 1 in our analysis have lived about 1.5 yr.

Despite our estimate of 30–35 cm for a 1.5– to 2-yr-old hake being in agreement with the results of others, a length above 30 cm for 1.5- to 2-yr-old hake seems large for a fish following von Bertalanffy growth with an expected T_0 near 0 yr of age. This suggests that hake growth in early life departs significantly from the von Bertalanffy form. Although such a departure is not uncommon (i.e. generally $T_0 \neq 0$) and might be expected, we must also caution that our hake collections taken off southern Vancouver Island will likely never adequately sample the smallest and youngest hake, thereby causing our model to overestimate the length of the youngest fish.

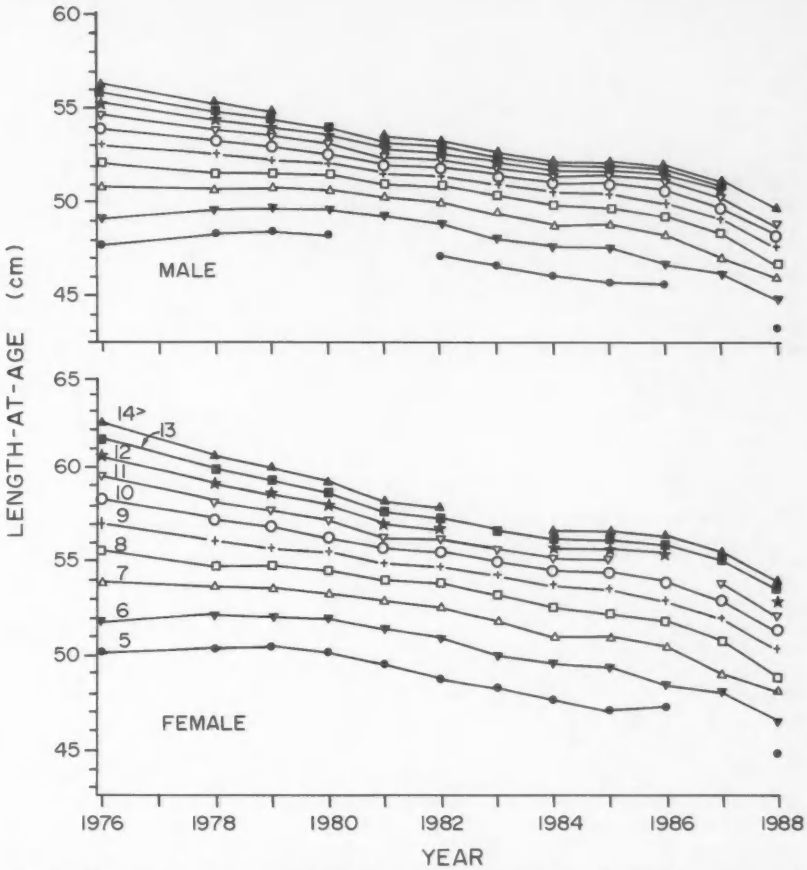

FIG. 8. Predicted pattern of mean lengths-at-age 5–14$^{>}$ using the maximum likelihood parameter estimates for four of the five processes proposed to describe the length-at-age pattern of male and female offshore Pacific hake sampled off southern Vancouver Island between July and October of 1976 until 1988. A fifth process, i.e. the influence of oceanographic factors in modifying the length-at-age distribution of hake in Canadian waters and incorporated into the predictions of Fig. 7, is excluded from these predictions.

Selectivity

Our results suggest that the fishery in Canadian waters is related to the declining trend in mean lengths-at-age of male and female hake by about -0.71 cm·yr^{-1} and -0.75 cm·yr^{-1}, respectively, for each percent of the estimated population biomass taken by the fishery in Canadian waters. For females this implies a length-selective mortality rate in 1988, the year of the largest catch from Canadian waters after 1976 ($C_{1988} = 90.5$ kt) of about ϵ (C_{1988}/B_{1988}) divided by 10 to 20 cm^2 (the approximate age-class variance for ages 5–14$^{>}$ from our unpublished length frequency distributions). The result of $(-74.9)(90.5)/1688$ yields about a 4 cm decrease in mean length-at-age with a corresponding instantaneous rate of length-selective mortality of about 0.2 to 0.4·cm^{-1}·yr^{-1}. Proportionally this means that, for example, a 45 cm female has a survival rate about 7–55 times higher than that of a 55 cm female. These rates are possibly excessive considering the 1988 fishery in Canadian waters exploited only about 5% of the hake biomass; however, because of age and length segregation, this fishery exploited a much larger percentage of the older age-classes and larger fish. Also, the 1988 predicted lengths-at-age underestimate the 1988 observed lengths-at-age for both males and females by about 2–3 cm (Fig. 9), therefore the 1988 observed lengths-at-age do not support the extreme length-selective mortality rates estimated from C_{1988}/B_{1988}.

Nevertheless, we don't encourage literal interpretation of the parameter estimates for ϵ, partly because the B_y's on which the estimates of ϵ (as well as δ and λ) are based have an associated estimation error and there is not absolute statistical independence between the estimates for B_y and our analysis. The stock synthesis model (Methot 1989) which generates the estimates for B_y does not include our data but it does include some assumptions about growth variability and length-selective fishing which conceivably could be recovered in our analysis. However, removing the effects of such processes from our length-at-age time series allows a more accurate interpretation of the effects of fishing in Canadian waters and oceanography. We also point out that length-selective fisheries off Oregon and Washington could potentially modify population lengths-at-

TABLE 4. Estimates and 95% confidence intervals (C.I.) for parameters of a model proposed to describe the length-at-age pattern of male and female offshore Pacific hake sampled off southern Vancouver Island between July and October of 1976 until 1988.

	Male		Female	
Parameters for:	Estimate	C.I.	Estimate	C.I.
von Bertalanffy growth				
$L_{5,1976}$	47.5	46.9 to 48.0	49.3	48.9 to 49.8
L_∞	60.0	59.2 to 60.7	65.7	64.7 to 66.7
K	0.18	0.15 to 0.20	0.17	0.14 to 0.19
Density-dependent growth				
$\delta \cdot 10^{-3}$	−1.03	1.22 to −0.84	−0.81	−0.95 to −0.68
General length-selectivity				
λ	5.15	4.22 to 6.07	5.35	4.66 to 6.04
Selective Canadian fishery				
ϵ	−70.5	−79.7 to −61.2	−74.9	−83.0 to −66.8
Oceanography				
β_1	−0.024	−0.029 to −0.020	−0.028	−0.031 to −0.024
β_2	0.16	0.12 to 0.19	0.14	0.11 to 0.17
β_3	−0.13	−0.17 to −0.10	−0.18	−0.21 to −0.15

TABLE 5. Correlation matrix of parameter estimates for a model proposed to describe the length-at-age patterns for male (upper triangle) and female (lower triangle) offshore Pacific hake sampled off southern Vancouver Island between July and October of 1976 until 1988.

	$L_{5,1976}$	L_∞	K	δ	ϵ	λ	β_1	β_2	β_3
$L_{5,1976}$	**1.00**	−0.29	0.18	0.77	−0.10	−0.77	0.21	−0.20	0.11
L_∞	−0.26	**1.00**	−0.83	−0.58	0.60	0.07	0.12	−0.12	0.20
K	0.22	−0.90	**1.00**	0.58	−0.85	0.19	−0.16	0.12	−0.23
δ	0.74	−0.48	0.52	**1.00**	−0.41	−0.63	0.07	−0.08	−0.14
ϵ	−0.21	0.74	−0.88	−0.38	**1.00**	−0.28	0.56	−0.49	0.52
λ	−0.68	−0.10	0.17	−0.70	−0.24	**1.00**	−0.27	0.27	−0.14
β_1	0.15	0.31	−0.24	0.05	0.51	−0.24	**1.00**	−0.86	0.78
β_2	−0.16	−0.31	0.20	−0.09	−0.40	0.34	−0.74	**1.00**	−0.57
β_3	0.00	0.29	−0.27	−0.27	0.45	0.08	0.65	−0.33	**1.00**

age, yet we do not include information from these fisheries in our model structure.

Consequently, we restrict our interpretation of the effects of fishing to the statement that the fishery in Canadian waters is a plausible explanation for the observed decline in mean lengths-at-ages 5–14$^>$ from 1976 until 1988. The decline arises from this fishery selectively removing the largest fish from the offshore hake population each year at a time when the population is length-segregated by migration. Similarly, the general length-selective mortality rate affecting population mean lengths-at-age in year y and related to $\log_e(B_y/B_{1976})$ by λ presumably captures the effects of both natural mortality and fishing mortality inflicted by the fisheries in American and Canadian waters. The estimates for λ indicate that in 1981, the year with the minimum B_y (Fig. 4), mean lengths-at-age were about 2 cm less than those in 1976, while in 1965, the year with the maximum B_y, they were about 3 cm more than in 1976. These extreme values for variability in population lengths-at-age are plausible and are less than those predicted to result from a length-selective fishery in Canadian waters.

Oceanography

The oceanographic covariate series of cumulative mean monthly anomalies of sea level height (Ω_y), surface temperature (Θ_y), and surface salinity (Φ_y) relate well to the interannual variability in mean lengths-at-age. Comparison of Fig. 7 with Fig. 8 reveals that oceanographic processes appear to be responsible for the characteristic interannual pattern in the mean length-at-age time series superimposed over the general decline. Of the three oceanographic covariate series, sea level height anomalies had the strongest relationship to interannual variability in mean lengths-at-age ($r^2 = 0.095$ and 0.074 for males and females, respectively). The negative relationship between cumulative monthly sea level height anomalies (Fig. 5) and mean lengths-at-age ($\beta_1 < 0$, Table 5) is consistent with a number of oceanographic studies which associate increased northward (or decreased southward) flow of coastal surface currents with above normal coastal sea level height, i.e. flow rates and sea level heights are in geostrophic balance (Smith 1974; Reid and Mantyla 1976; Huyer et al. 1978; McLain and Thomas 1983; Emery and Hamilton 1985).

As is typical of multiple regression analysis, the positive ($\beta_2 > 0$, Table 5) relationship between cumulative monthly surface temperature anomalies and mean lengths-at-age result from (1) this covariate series being less strongly negatively related to the interannual change in mean lengths-at-age ($r^2 = 0.042$ and 0.026 for males and females, respectively) than sea level height anomalies, and (2) sea level height anomalies being positively correlated with surface temperature anomalies (Fig. 11). A similar, but reverse, explanation applies to the negative relationship ($\beta_3 < 0$, Table 5) between sea surface salinity and interannual change in mean lengths-at-age ($r^2 = 0.036$ and 0.031 for males and females, respectively).

In summary then, higher than normal sea level height and surface temperature anomalies, and lower than normal surface salinity anomalies are associated with lower than expectd mean

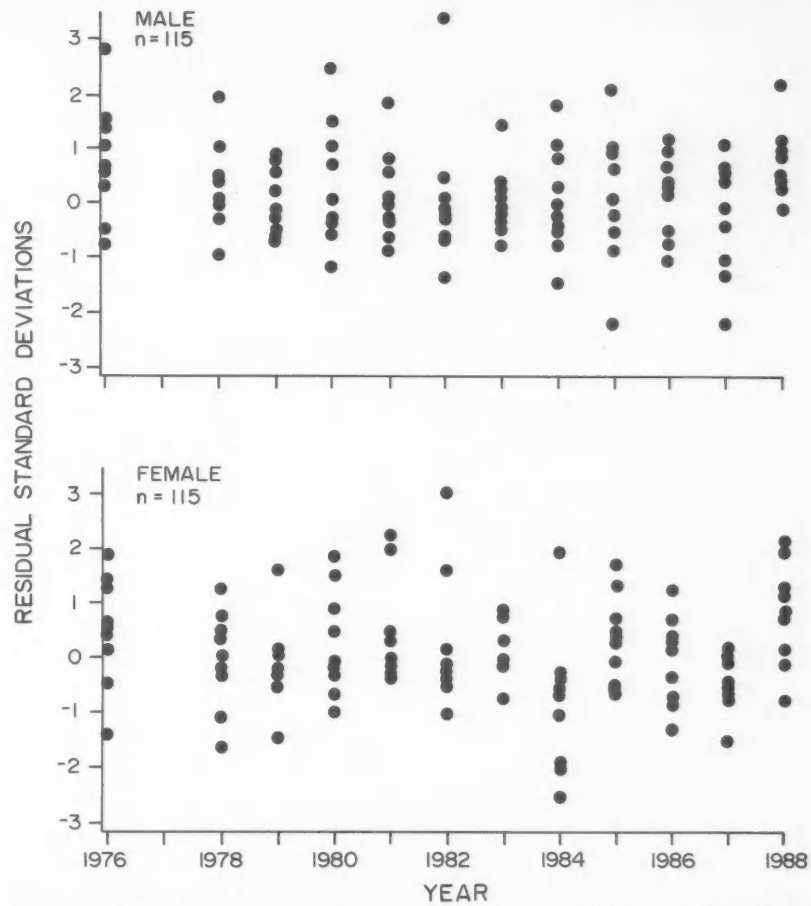

FIG. 9. Distribution of standardized residuals of the length-at-age predictions, $(S_{i,y} - O_{i,y})/MS^{1/2}$, vs. the years sampled from 1976 until 1988. MS is the mean squared residual error from Table 3.

lengths-at-ages for any year. Surface salinity anomalies are lower than normal, and surface temperature anomalies are higher than normal, in years of strong northward (or weak southward) flowing coastal surface currents due to less upwelling of higher salinity subsurface water (Reid and Mantyla 1976; Dodimead 1984). Periods of anomalously low upwelling occur during periods of weaker than normal southerly winds (decreased offshore Ekman transport) and are associated with stronger northward (or weaker southward) flows of coastal surface currents. Thus, we interpret that the northward migration of hake is assisted in years of a stronger than normal northward (or weaker than normal southward) flow resulting in more smaller hake (of any age-class) arriving off southern Vancouver Island. Comparison of Fig. 7 with Fig. 8 indicates that oceanographic factors appear to be capable of modifying hake mean lengths-at-age off southern Vancouver Island by 2–3 cm among years.

It is worthy of note at this point that preliminary model structures which included the oceanographic data as covariate series related directly to real growth rates yielded minimum variances inferior to the model described herein, where the oceanographic data are related only to the length distribution of hake in Canadian waters. This implies that the oceanographic time series, as incorporated into our model, are not revealing about related environmental (e.g. growth rates vs. temperature) or biological (e.g. growth rates vs. food availability) factors which are in fact likely to affect real growth rates in some way.

Discussion

We conclude that all of (1) growth rate variability, (2) length-selective total mortality, (3) length-selectivity by the fishery in Canadian waters upon a hake population length-segregated by migration, and (4) oceanographic conditions bear significant statistical relationship to the observed length-at-age pattern for offshore Pacific hake sampled off Vancouver Island from 1976 until 1988. We acknowledge that the statistical evidence for density-dependent growth and general length-selective mortality might result partly from the reconstructed population biomass estimates (B_y); however, the statistical evidence that the fishery in Canadian waters has steadily decreased population mean lengths-at-age $5\text{–}14^{>}$ since 1976 is sound. We are also confident that population mean lengths-at-age are not accurately represented by our sample mean lengths-at-age because oceanographic factors modify the interannual length and age

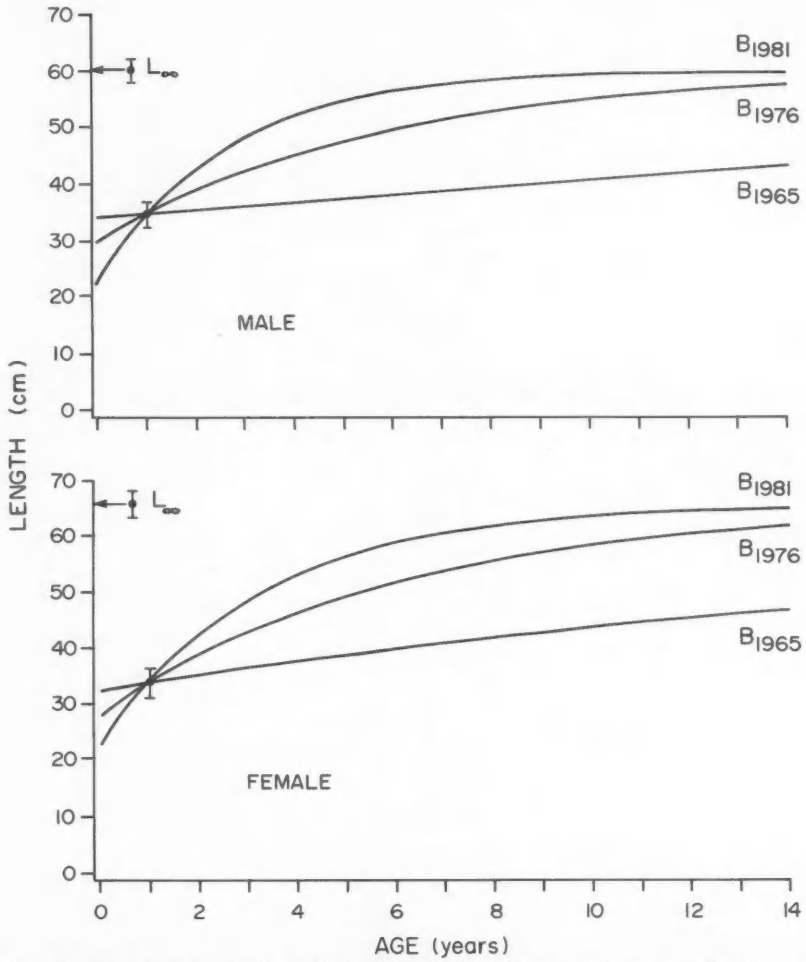

FIG. 10. Predicted von Bertalanffy growth functions related to offshore Pacific hake population biomass for the extreme values of B_y. Maximum B_y of 3695 kt occurred in 1965 and the minimum B_y of 1384 kt occurred in 1981. All parameter estimates were obtained with rate processes standardized to B_{1976} (2055 kt). For $L_{1,y}$ and L_∞ the 95% C.L. are shown.

distributions of offshore Pacific hake off Vancouver Island during summer.

Variation in population length-at-age structure due to length-dependent, or perhaps more appropriately growth rate-dependent, natural and fishing mortality rates is generally accepted as a natural process in many populations (Ricker 1969). However, our study is among only a few (notably Ricker (1981)) which statistically support the hypothesis that length-selective fishing can substantially modify population length-at-age structure. We believe our study is unique in attributing changes in population mean lengths-at-age to differential fishing rates upon a gadoid population well documented to be length-segregated by migration (Francis 1983, Beamish and McFarlane 1985, Nelson and Dark 1985).

In support of our conclusion that oceanographic factors modify sampled lengths-at-age, the high abundance of 3-yr old hake off southern Vancouver Island during the ENSO year of 1983 (Fig. 2, 3) was unusual, despite the strength of the 1980 year-class. This specific event supports our analytical conclusion that more smaller hake, and consequently a greater biomass of hake, arrive in Canadian waters in years of above normal northward (or below normal southward) surface flow. Anomalously strong northward coastal surface flow during the 1983 ENSO year (see Fig. 11 and Mysak (1986)) probably greatly assisted the northward migration of these 3-yr old hake, and older hake as evidenced by the marked decrease in sampled lengths-at-age off Vancouver Island during 1983 (Fig. 6, 7).

We wish to emphasize that all of the modelled processes have the potential for generating sample lengths-at-age which do not accurately reflect the growth of individuals or populations. The presence of process 6 in our Introduction, sampling bias related to the age and length structure of Pacific hake schools, could not be statistically supported, but the visual pattern of mean lengths-at-age (Fig. 6) is compelling evidence for its existence

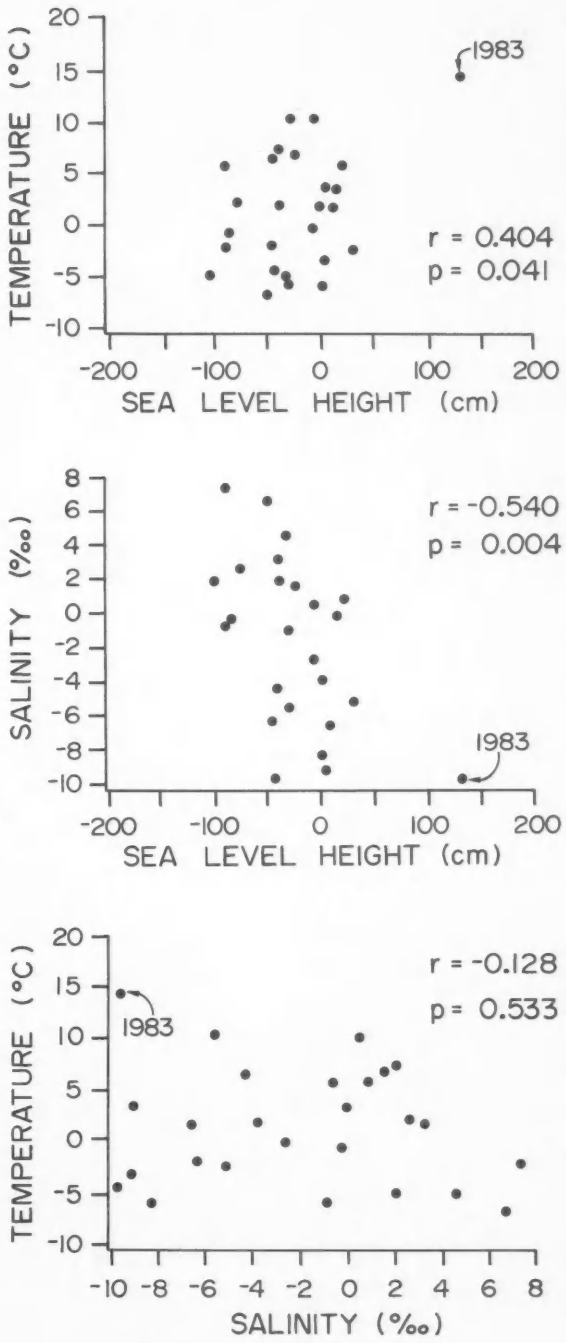

FIG. 11. Correlations between each pair of the oceanographic covariate series of cumulative mean monthly anomalies of sea level height (Ω_y), surface temperature (Θ_y) and surface salinity (Φ_y) for the 12 mo from August of year y-1 until July of year y beginning in year y = 1963. The data points for 1983, an ENSO year, are indicated.

in some of the years sampled. Welch and McFarlane (1990) demonstrate the existence of growth rate variability for Strait of Georgia hake and caution, as did Ricker (1969), that growth variability and parameter uncertainty should be considered in yield analyses. We similarly caution that population models, and consequently stock assessments, that do not address the dynamic processes that affect the growth of individuals and populations, or which result in sampling bias, also have the potential to be grossly in error with serious short-term and long-term negative consequences for management.

We have demonstrated that the processes we incorporated into our explanatory model have the potential to modify population mean lengths-at-age by as much as about 5 cm. Translated to a weight (yield) difference, where $W = \alpha L^{\gamma}$, a 5 cm bias in length-at-age for a 55 cm hake, for example, results in a $((L+5)/L)^{\gamma}$ weight differential. For values of γ for offshore hake near 2.7 (Francis 1983) this results in a very serious error in estimated weight of about 26%. Errors of such magnitude would be seriously detrimental to fishery yield estimates. We therefore advise that growth rate estimates for offshore Pacific hake should be acknowledged as conditional on population status, fishery selectivity, and oceanographic conditions. For example, we conditioned our growth parameter estimates on the assumption that 1976 is a year with no length-selective biases, and that there is density dependence in growth rates. Our choice of 1976 as the year with neutral length-selective biases in population length-at-age is arbitrary, and we have no experimental evidence for the existence of density dependent growth. However, we plan to investigate interannual variation in otolith growth patterns for evidence of growth variability related to population or oceanographic covariate series.

The discovery of a statistical relationship between the distribution and abundance of offshore Pacific hake in Canadian waters and oceanographic factors contributes to previous work by others which has related oceanographic conditions to year-class strength of offshore Pacific hake. Pacific hake eggs, larvae, and juveniles have been successfully sampled with the result that Bailey (1981) and Hollowed and Bailey (1989) were able to infer some influences of oceanography on potential year-class strength. Bailey (1980) also proposed a link between a northern shift in abundance of hake larvae off southern California during the mid-1960's and an intensification of the fishery for large hake during summer off the coasts of Oregon and Washington. Bailey (1980) hypothesized that selectively removing the largest hake near the northern extent of their migration removed those individuals which would migrate furthest south to spawn. Bailey's (1980) proposal is closely linked conceptually with the hypothesis of Beamish (1981) and Beamish and McFarlane (1985) that the fishery in Canadian waters has the potential to seriously reduce population egg production because it captures large, mature, fecund females.

In addition to the possible reduction in population egg production over the longer term, the short-term implications of transboundary length segregation also needs further study. Our results support the contention that despite <1–36% of the total annual catch from 1976 until 1988 occurring in Canadian waters, this fishery can be sufficiently intense to affect its short-term future by selectively removing those hake large enough to migrate as far north as southern Vancouver Island during the following few years. Clearly then, our results generate some predictions, notably that the fishery in Canadian waters and oceanographic conditions can affect the future transboundary distribution of Pacific hake. This is a major international man-

agement problem and we must continue to quantify the effects of the processes identified in this study on the transboundary distribution of offshore Pacific hake. We should also continue to closely monitor trends in hake abundance and length-at-age for both indications of, and the effects of, long-term oceanographic climate change.

Acknowledgments

We are grateful to the technical staff at the Pacific Biological Station and the on-board observers who were responsible for collecting most of our Pacific hake samples, and to Ms. S. MacLellan for ageing the samples. This manuscript was improved through criticisms by Drs. A. Tyler and D. Ware of PBS, while the oceanographic arguments were sharpened by Dr. R. Thomson of the Institute of Ocean Sciences, Sidney, British Columbia. We also thank Dr. R. Thomson for making available to us the up-to-date time series of sea level height, temperature, and salinity records for Amphitrite Point and Tofino, British Columbia.

References

BAILEY, K. M. 1980. Recent changes in the distribution of hake larvae: causes and consequences. Calif. Coop. Oceanic Fish. Invest. Rep. 21: 167–171.

1981. Larval transport and recruitment of Pacific hake, *Merluccius productus*. Mar. Ecol. Prog. Ser. 6: 1–9.

BAILEY, K. M., AND R. C. FRANCIS. 1985. Recruitment of Pacific whiting, *Merluccius productus*, and the ocean environment. Mar. Fish. Rev. 45: 8–15.

BAILEY, K. M., R. C. FRANCIS, AND P. R. STEVENS. 1982. The life history and fishery of Pacific whiting *Merluccius productus*. Calif. Coop. Oceanic Fish. Invest. Rep. 23: 81–98.

BARNER, L. W., R. KEISER, AND T. J. MULLIGAN. 1984. A hydroacoustic survey for Pacific hake on the continental shelf off British Columbia and Washington from 48 to 49 degrees north latitude: August 22 to September 8, 1983. Can. Data Rep. Fish. Aquat. Sci. 458: 98 p.

BEAMISH, R. J. 1979. Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. J. Fish. Res. Board Can. 36: 141–151.

1981. A preliminary report of Pacific hake studies conducted off the west coast of Vancouver Island. Can. Manuscr. Rep. Fish. Aquat. Sci. 1610: 43 p.

BEAMISH, R. J., AND G. A. MCFARLANE. 1985. Pacific whiting, *Merluccius productus*, stocks off the west coast of Vancouver Island, Canada. Mar. Fish. Rev. 47: 75–81.

BEAMISH, R. J., G. A. MCFARLANE, K. R. WEIR, M. S. SMITH, J. R. SCARSBROOK, A. J. CASS, AND C. C. WOOD. 1982. Observations on the biology of Pacific hake, walleye pollock and spiny dogfish in the Strait of Georgia, Juan de Fuca Strait and off the west coast of Vancouver Island and the United States. *Arctic Harvester*, July 13–24, 1976. Can. Manuscr. Rep. Fish. Aquat. Sci. 1651: 150 p.

CHELTON, D. B., AND R. E. DAVIS. 1982. Monthly mean sea-level variability along the west coast of North America. J. Phys. Oceanogr. 12: 757–784.

DAVENPORT, D. 1985. Biological observations of the foreign hake fishery. Can. Manuscr. Rep. Fish. Aquat. Sci. 1811: 15 p.

DODIMEAD, A. J. 1984. A review of some aspects of the physical oceanography of the continental shelf and slope waters off the west coast of Vancouver Island, British Columbia. Can. Manuscr. Rep. Fish. Aquat. Sci. 1773: 309 p.

EMERY, W. J., AND K. HAMILTON. 1985. Atmospheric forcing of interannual variability in the northeast Pacific Ocean: connections with El Niño. J. Geophys. Res. 90: 857–868.

FAVORITE, F., A. J. DODIMEAD, AND K. NASU. 1976. Oceanography of the subarctic Pacific region, 1960–71. Int. N. Pac. Fish. Comm. Bull. 33: 187 p.

FRANCIS, R. C. 1983. Population and trophic dynamics of Pacific hake (*Merluccius productus*). Can. J. Fish. Aquat. Sci. 40: 1925–1943.

FREELAND, H. J., W. R. CRAWFORD, AND R. E. THOMSON. 1984. Currents along the Pacific coast of Canada. Atmosphere-Ocean 22: 151–172.

HICKEY, B. M. 1979. The California Current system – hypotheses and fact. Prog. Oceanogr. 8: 191–279.

HOLLOWED, A. B., AND K. M. BAILEY. 1989. New perspectives on the relationship between recruitment of Pacific hake *Merluccius productus* and the ocean environment, p. 207–220. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. Can. Spec. Pub. Fish. Aquat. Sci. 108 p.

HUYER, A., R. L. SMITH, AND E. J. C. SOBEY. 1978. Seasonal differences in low-frequency current fluctuations over the Oregon continental shelf. J. Geophys. Res. 83: 5077–5089.

JONES, R. 1958. Lees's phenomenon of "apparent change in growth-rate" with particular reference to haddock and plaice. Int. Comm. Northwest Atl. Fish. Spec. Publ. 1: 229–242.

KABATA, Z., AND D. J. WHITAKER. 1981. Two species of *Kudoa* (Myxosporea: Multivalvulida) parasitic in the flesh of *Merluccius productus* (Ayres, 1855) (Pisces: Teleostei) in the Canadian Pacific. Can. J. Zool. 59: 2085–2091.

1986. Distribution of two species of *Kudoa* (Myxozoa: Multivalvulida) in the offshore population of Pacific hake, *Merluccius productus* (Ayres, 1855). Can. J. Zool. 64: 2103–2110.

KENDALL, M., AND A. STUART. 1979. The advanced theory of statistics, Vol. 2. Inference and relationship, 4th ed. MacMillan Publishing Company, New York, NY. 748 p.

LEE, R. M. 1912. An investigation into the methods of growth determination in fishes. Publ. Circonstance, Cons. Perm. Explor. Mer 63 p.

LYNN, R. J., AND J. J. SIMPSON. 1987. The California current system: the seasonal variability of its physical characteristics. J. Geophys. Res. 92: 12947–12966.

MCFARLANE, G. A., AND R. J. BEAMISH. 1985. Biology and fishery of Pacific whiting, *Merluccius productus*, in the Strait of Georgia. Mar. Fish. Rev. 47: 23–34.

MCLAIN, D. R., AND D. H. THOMAS. 1983. Year-to-year fluctuations of the California countercurrent and effects on marine organisms. Calif. Coop. Oceanic Fish. Invest. Rep. 24: 165–181.

METHOT, R. D. 1989. Synthetic estimates of historical abundance and mortality for northern anchovy, p. 66–82. *In* E. F. Edwards and B. A. Megrey [ed.] Mathematical analysis of fish stock dynamics. Am. Fish. Soc. Sym. 6.

MITTERTREINER, A., AND J. SCHNUTE. 1985. Simplex: a manual and software package for easy nonlinear parameter estimation and interpretation in fishery research. Can. Tech. Rep. Fish. Aquat. Sci. 1384: 90 p.

MYSAK, L. A. 1986. El Niño, interannual variability and fisheries in the northeast Pacific Ocean. Can. J. Fish. Aquat. Sci. 4: 464–497.

NELDER, J. A., AND R. MEAD. 1965. A simplex method for function minimization. Comput. J. 7: 308–313.

NELSON, M. O., AND T. A. DARK. 1985. Results of the coastal Pacific whiting, *Merluccius productus*, surveys in 1977 and 1980. Mar. Fish. Rev. 47: 82–94.

PEDERSEN, M. 1985. Puget Sound Pacific whiting, *Merluccius productus*, resource and industry: an overview. Mar. Fish. Rev. 47: 35–38.

REID, J. L., AND A. W. MANTYLA. 1976. The effect of geostrophic flow upon coastal sea elevations in the northern North Pacific Ocean J. Geophys. Res. 81: 3100–3110.

RICKER, W. E. 1969. Effects of size-selective mortality and sampling bias on estimates of growth, mortality, production, and yield. J. Fish. Res. Board Can. 26: 479–541.

1981. Changes in the average size and average age of Pacific salmon. Can. J. Fish. Aquat. Sci. 38: 1636–1656.

SAUNDERS, M. W., AND W. SHAW. 1989. Pacific hake, p. 133–167. *In* J. Fargo and A. V. Tyler [ed.] Groundfish stock assessments for the west coast of Canada in 1988 and recommended yield options for 1989. Can. Tech. Rep. Fish. Aquat. Sci. 1646: 294 p.

SCHNUTE, J. 1981. A versatile growth model with statistically stable parameters. Can. J. Fish. Aquat. Sci. 38: 1128–1140.

SHAW, W., G. A. MCFARLANE, J. R. SCARSBROOK, M. S. SMITH, AND W. T. ANDREWS. 1985. Distribution and biology of Pacific hake and walleye pollock off the west coast of Vancouver Island and the state of Washington. (August 15–September 5, 1983). Can. Manuscr. Rep. Fish. Aquat. Sci. 1825: 128 p.

SHAW, W., R. TANASICHUK, D. M. WARE, D. DAVENPORT, AND G. A. MCFARLANE. 1987. Biological and species interaction survey of Pacific hake, sablefish, spiny dogfish and Pacific herring off the southwest coast of Vancouver Island. F/V *Eastward Ho*, August 10–22, 1985. Can. Data Rep. Fish. Aquat. Sci. 651: 49 p.

SHAW, W., R. TANASICHUK, D. M. WARE, AND G. A. MCFARLANE. 1989. Biological and species interaction survey of Pacific hake, sablefish, spiny dogfish and Pacific herring off the southwest coast of Vancouver Island. F/V *Caledonian*, August 12–25, 1986. Can. Manuscr. Rep. Fish. Aquat. Sci. 2012: 134 p.

SMITH, R. L. 1974. A description of current, wind, and sea level variations during coastal upwelling off the Oregon coast, July–August 1972. J. Geophys. Res. 79: 435–443.

STAUFFER, G. D. 1985. Biology and life history of the coastal stock of Pacific whiting, *Merluccius productus*. Mar. Fish. Rev. 47: 2–7.

SWARTZMAN, G. L., W. M. GETZ, AND R. C. FRANCIS. 1987. Binational management of Pacific hake (*Merluccius productus*): a stochastic modeling approach. Can. J. Fish. Aquat. Sci. 44: 1053–1063.

TABATA, S. 1975. The general circulation of the Pacific Ocean and a brief account of the oceanographic structure of the North Pacific Ocean. Atmosphere 13: 133–168.

THOMSON, R. E. 1981. Oceanography of the British Columbia coast. Can. Spec. Pub. Fish. Aquat. Sci. 56: 291 p.

THOMSON, R. E., AND D. M. WARE. 1988. Oceanic factors affecting the distribution and recruitment of west coast fisheries, p. 31–65. *In* M. Sinclair, J. T. Anderson, M. Chadwick, J. Gagné, W. D. McKone, J. C. Rice, and D. Ware [ed.] Report from the national workshop on recruitment. Can. Tech. Rep. Fish. Aquat. Sci. 1626: 261 p.

THOMSON, R. E., B. M. HICKEY, AND P. H. LEBLOND. 1989. The Vancouver Island Coastal Current: fisheries barrier and conduit, p. 265–296. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. Can. Spec. Pub. Fish. Aquat. Sci. 108 p.

WARE, D. M. 1978. Bioenergetics of pelagic fish: theoretical change in swimming speed and ration with body size. J. Fish. Res. Board Can. 35: 220–228.

WARE, D. M., AND G. A. MCFARLANE. 1989. Fisheries production domains in the Northeast Pacific Ocean, p. 359–379. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. Can. Spec. Pub. Fish. Aquat. Sci. 108 p.

WEIHS, D., AND P. W. WEBB. 1983. Optimization of locomotion, p. 339–371, Chap. 11. *In* D. Weihs and P. W. Webb [ed.] Fish Biomechanics. Praeger, New York, NY.

WELCH, D. W., AND G. A. MCFARLANE. 1990. Quantifying the growth of female Pacific hake (*Merluccius productus*): an example of measuring uncertainty and bias in non-linear parameter estimation. Can. J. Fish. Aquat. Sci. 47: 672–681.

ZAR, Z. H. 1984. Biostatistical analysis, 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey.

Growth of Juvenile (0-Group) Atlantic Mackerel (*Scomber scombrus*) in the Gulf of St. Lawrence

Denis D'Amours and Jean G. Landry

Division de la Recherche sur les Pêches, Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli, Québec G5H 3Z4 Canada

and Timothy C. Lambert

Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2 Canada

D'Amours, D., J. G. Landry, and T. C. Lambert. 1990. Growth of juvenile (0-group) Atlantic mackerel (*Scomber scombrus*) in the Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 47: 2212–2218.

Juvenile (0-group) Atlantic mackerel were captured between 1977 and 1980, and in 1988, in the Gulf of St. Lawrence. Daily rings were identified and validated on their sagittae over the size range 93–170 mm; the age of larval and juvenile mackerel was estimated over the size range 7–192 mm (fork length). A one-cycle Gompertz curve was fitted to length-at-age data and the parameters were: L_∞ = 180.8 mm, K = 0.043, and t_o = 37.7 d. The maximum calculated growth rate was 2.86 mm/d. The growth rate of 0-yr mackerel from the Gulf of St. Lawrence could be faster from that reported for 0-yr mackerel from the Middle Atlantic Bight: the mean growth rate of fish from both regions was respectively 1.38 and 1.02 mm/d in the age interval 10–20 d; 2.30 and 2.07 mm/d in the age interval 20–30 d; and 2.79 and 2.69 mm/d in the age interval 30–40 d. This difference in somatic growth rate could provide the basis for determining the origin of adult fish if it resulted in permanent and discernible records on the otoliths, and if otolith growth is coupled with somatic growth.

Des maquereaux bleus juvéniles (groupe 0) ont été capturés entre 1977 et 1980 et en 1988 dans le golfe du Saint-Laurent. Des anneaux quotidiens ont été repérés et validés sur les sagittae de ces poissons (93–170 mm); l'âge de larves et de juvéniles a été évalué sur des spécimens dans l'intervalle de taille 7–192 mm. Une courbe de Gompertz à un cycle a été adaptée pour les données sur la longueur en fonction de l'âge; les paramètres étaient L_∞ = 180.8 mm, K = 0.043 et t_o = 37.7 j. Le taux de croissance maximal calculé était de 2.86 mm/j. Le rythme de croissance des maquereaux du groupe 0 du golfe du Saint-Laurent pourrait être plus rapide que le taux signalé pour les maquereaux du groupe 0 de la Middle Atlantic Bight : le taux moyen de croissance des poissons de ces deux régions était respectivement de 1.38 et de 1.02 mm/j pour l'intervalle d'âge de 10–20 j, 2.30 et 2.07 mm/j pour l'intervalle d'âge de 20–30 j et 2.79 et 2.69 mm/j pour l'intervalle d'âge de 30–40 j. Cette différence du taux de croissance somatique pourrait fournir la base à partir de laquelle on pourrait déterminer l'origine des poissons adultes si elle résultait en des registres permanents et détectables sur les otolithes et si la croissance des otolithes était jumelée à la croissance somatique.

Received October 10, 1989
Accepted May 10, 1990
(JA336)

Reçu le 10 octobre 1989
Accepté le 10 mai 1990

The population of Northwest Atlantic mackerel (*Scomber scombrus*) is classically thought to be composed of a northern contingent, spawning in the Gulf of St. Lawrence, and a southern contingent, spawning in the Middle Atlantic Bight (Sette 1943, 1950; Berrien 1978). Individuals from the northern contingent spawn mainly in late June (Ware 1977), and their progeny will reach 200 mm fork length at the end of November of the same year (MacKay 1979; Ware and Lambert 1985). Fish from the southern contingent spawn in late April or early May (Morse 1980). Despite the earlier spawning of the southern fish relative to the northern ones, their progeny appear to reach the same terminal length of 200 mm at the end of their first growing season (Sette 1950). Because of this sizable lag in spawning times, with similar terminal lengths, a difference in growth rate is to be expected between the juvenile fish of each contingent. As early as 1950, it was recognized by Sette that an investigation of this growth differential was a promising approach for studying the exchange between the two contingents of mackerel. The difference in growth rate at the juvenile stage, if it resulted in permanent record on growth structures, could provide the basis for identifying the contingent of origin of mackerel. However, the growth of juvenile mackerel during this first growing season, in either contingent, is yet to be described in detail.

The available accounts on the growth of juvenile mackerel are either based on incidental captures, or span only a fraction of the size range of the fish during its first growing season. Kendall and Gordon (1981) have described the growth of larval and juvenile mackerel from the southern contingent of the northwest Atlantic population in the Middle Atlantic Bight. These authors based their analysis of growth on size-at-age, as determined with growth rings on the otoliths of the fish. However, the growth curve they derived is only valid for fish up to a size of 70 mm (SL). Further, Kendall and Gordon (1981) have not produced a direct or indirect validation of their ageing technique. Such an unvalidated interpretation of growth rings makes the results provisional (see Beamish and McFarlane 1983). Another description of the growth of juvenile mackerel

was made by Ware and Lambert (1985). They produced a growth curve spanning most of the size range of juvenile mackerel in their first growing season. Ware and Lambert (1985) used length-at-date to derive the growth curve, since the egg release of Atlantic mackerel may be described as an "all at once type" (Lambert and Ware 1984). However, the degree of correspondence between age and date assumed in this model remains to be verified.

In this paper, the growth of juvenile Atlantic mackerel during their first growing season in the Gulf of St. Lawrence is described. The objectives of the study are to determine whether the microstructure on the otolith of juvenile mackerel could be used to age young fish reliably, and to describe the growth of 0-group fish with length-at-age over the whole range of size of fish during their first growing season. The results are then discussed relative to the available information on the growth of juvenile mackerel from the Middle Atlantic Bight.

Study Site and Methods

Larval and juvenile mackerel were captured in the Gulf of St. Lawrence near Shippegan, New Brunswick (Fig. 1) in July and August 1988. Juvenile mackerel were captured with a beach seine or with gillnets in nearshore water; larval fish were captured during surface hauls about 6 km offshore between July 18 and August 3, with a standard plankton net (500 μm). A detailed account of the capture techniques and equipment used is provided by D'Amours and Landry (1989). Other juvenile mackerel were captured in traps, smelt gillnets or with lines and hooks in St. Georges Bay, Nova Scotia from 1977 to 1980 (see Ware and Lambert 1985 for details of the capture).

For the validation experiment, some of the fish captured with the beach seine in 1988 were maintained alive at the Aquarium and Marine Center in Shippegan (Fig. 1) in a 2000 L holding tank, with a continuous water flow of 10 L/min. The water temperature in the holding tank ranged from 20°C on August 8 to 16°C on September 4, with a salinity of about 27 ppt. From August 9 to August 11, a total of 115 juvenile mackerel, ranging in size from 80 to 95 mm, were stocked in the holding tank. On August 11, all the detained fish were transferred to an insulated tank containing 200 L of water with a salinity of 25 ppt and a temperature of 20°C, to which calcein (2,4-bis-[N,N'-di(carboxymethyl)-amino-methyl]fluorescein) (Wilson et al. 1987) was added from a stock solution containing 4.16 g calcein/L of distilled water. The final concentration of calcein in the tank was 125 mg/L. The fish were maintained in the tank for 2 h, while the water was aerated with bubbling stones, and transferred back to the 2000 L holding tank. The captive fish were fed chopped herring dispensed in three equal portions at 0800, 1600, and 2200. To ensure good growth, the total daily ration of about 24% wet body weight, was well above the 1–2% maintenance level required for adult mackerel (T. C. Lambert, unpubl. data). The light cycle in the room was 10 hr L/14 hr D; when feeding the fish at night, the tank was indirectly illuminated with a fluorescent light for the time of feeding. Every third day after marking the fish with calcein, 6–10 fish were removed from the detention tank (see D'Amours and Landry 1989 for a discussion of husbandry techniques). The sagittae of the fish were extracted, mounted in cyanoacrylate (Krazy Glue™) on a microscope slide, and kept in the dark. The fluorescent marks on the otolith of the fish treated with calcein were observed under reflected light from a 100W mercury lamp and through a filter with an excitation range in the ultra-violet (340–380 nm half power points).

The age of juvenile fish was determined on specimens captured in Shippegan and in St. Georges Bay. The fish caught in Shippegan were preserved in anhydrous ethanol; a factor of +4% was used to correct length measured on preserved fish (D'Amours and Landry 1989). After 2–3 mo in alcohol, the sagittae were extracted and mounted in cyanoacrylate. For the fish captured in St. Georges Bay, the sagittae were extracted from fresh fish and kept dry until mounted in 1988.

All observed otoliths were ground on one side in the sagittal plane with respect to the fish (Summerfelt and Hall 1986, p. 527–530) with self-adhesive 3M Imperial Lapping Film (3 and 30 μm grit), as per Campana and Neilson (1985). Otoliths were observed with a microscope coupled to an image analysis system. The central part of the otolith (usually the first 15 to 20 rings) was read at 1000× in immersion oil, while the rest of the otolith was read at 250× in interference contrast microscopy. Each otolith was read by two persons and the ring count was recorded only after an agreement was reached. The sagittae of the smaller fish (7–19 mm fork length) were read first at 1000× to obtain a clear image of the structure of the otherwise difficult to read central region (Fig. 2A). The enumeration of growth rings on the otoliths was easier inside two triangular surfaces pointing to the core in the dorso-ventral axis relative to the fish (Fig. 2B); these two surfaces were defined as the standard reading fields. The standard reading fields corresponded to the small axis of the otolith. Because the otoliths of mackerel are curved along the long axis, the number of rings along the long axis was difficult to estimate. No comparison was made between the numbers of rings on the long and short axes.

A one-cycle Gompertz curve (see Ricker 1973, page 705) was fitted to the length-at-age data for the combined samples from St. Georges Bay and from Shippegan. The model was:

$$(1)\quad L_t = L_\infty e^{-e^{-K(t-t_o)}}$$

where L_t = length in mm at age (in days), L_∞ = asymptotic fork length (mm), K = growth coefficient, and t_o = point of inflection (age of maximum growth). A weighting factor equal to the inverse of age was introduced in the least-squares analysis (Box et al. 1978) and the growth curve was parametrized by iteration following Marquardt's algorithm on SAS (SAS Institute Inc. 1985).

Results

Frequency of Deposition of Rings on the Otoliths of Captive Fish

The captive fish grew from means of 95 ± 4.7 mm and 8.1 ± 1.6 g to means of 144 ± 12.9 mm and 29.3 ± 7.8 g (fork length and wet weight, respectively), from August 14 to September 4. A stress check was observed on the otoliths of the calcein treated fish (Fig. 3A). This check was assumed to have resulted from the combined stress of capture, transfer, and immersion in the calcein bath. A thin blue fluorescent band was associated with this stress check (Fig. 3B). Other checks appeared on the otoliths under UV light, either along regular bands, or along fissures. However, only the check associated with the sharp stress check was a definite blue; the other checks visible in UV were white or yellowish. Furthermore, the otoliths of fish that had been kept captive, but not immersed in calcein,

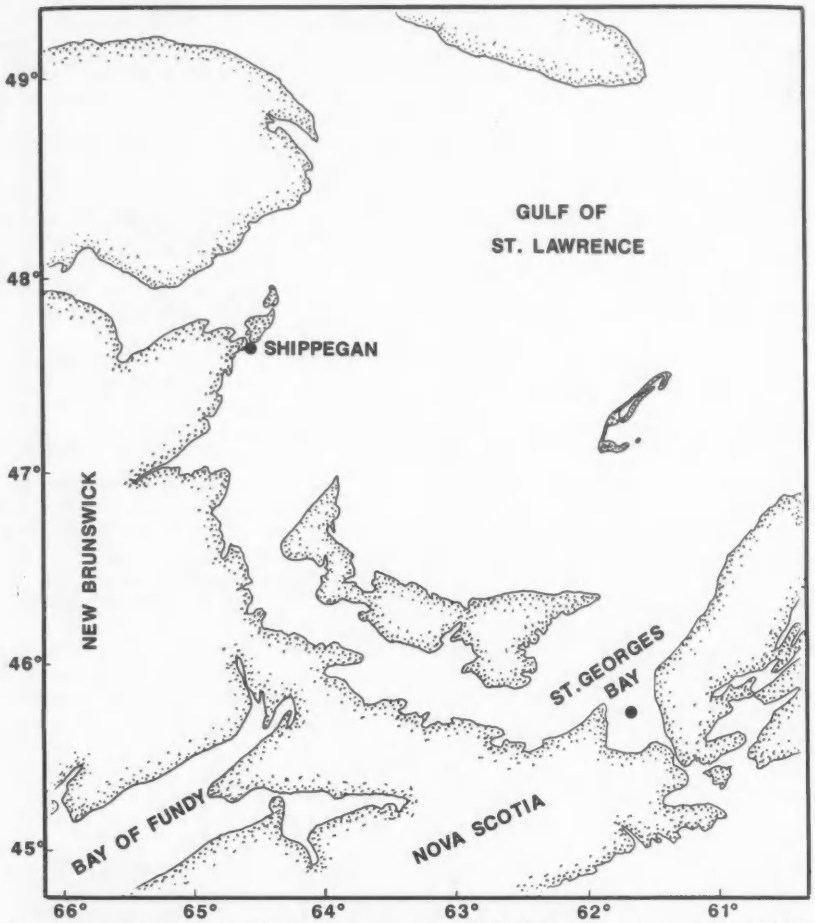

FIG. 1. Map of study site. Juvenile mackerel were captured in St. Georges Bay between 1977 and 1980; other juvenile and larval mackerel were captured near Shippegan in 1988.

did not show such a thin blue fluorescent band. Only the otoliths showing this thin blue fluorescent band inside the standard reading zones defined for the wild fish (see above) were kept for the validation process. As a result of this selection, the otoliths of 45% of the calcein treated fish were rejected. Beyond the blue fluorescent check visible in UV, sharp discontinuous zones were observed and enumerated in interference microscopy. The number of such zones was regressed against the number of days since immersion of fish in calcein. The regression equation was:

$$(2) \quad N = -0.14 + 1.03\,D$$

where N = number of sharp discontinuous zones after fluorescent band; D = number of days since immersion of the fish in calcein. The linear model was highly significant (R^2 = 0.99; P = 0.0001; n = 38), and the residuals showed no obvious patterns. The slope of Eq. 2 (1.03 with SE = 0.0146) was not significantly different from 1 (two-tailed t-test; $0.4>P>0.2$), and the intercept (−0.14 with SE = 0.24) was not significantly different from 0 (two-tailed t-test; P = 0.57). The growth rings identified on the otoliths of juvenile mackerel within the size range of the validation experiment, were thus conclusively identified as daily.

Growth of Wild Fish with Length-at-Age

On the Gompertz growth curve computed with length-at-age (Fig. 4), the asymptotic length (L_∞) was 180.8 mm; the growth coefficient (K), 0.043; and the inflection point (t_o), 37.7 d (Table 1). The 3-parameter growth model accounted for most of the variation in size with a highly significant ($P<0.001$) multiple coefficient of determination ($R^2=0.99$). The maximum growth of juvenile mackerel was 2.86 mm/d between age 37.7 d (t_o) and age 38.7 d. The mean growth rate was 1.38 mm/d over the age interval 10–20 days; 2.30 mm/d over the interval 20–30 days; and 2.79 mm/d over the interval 30–40 days.

Discussion

Growth Rings as Age Record

Initially, it was not obvious which pattern of rings should be used to determine age. On the same otolith, variable counts

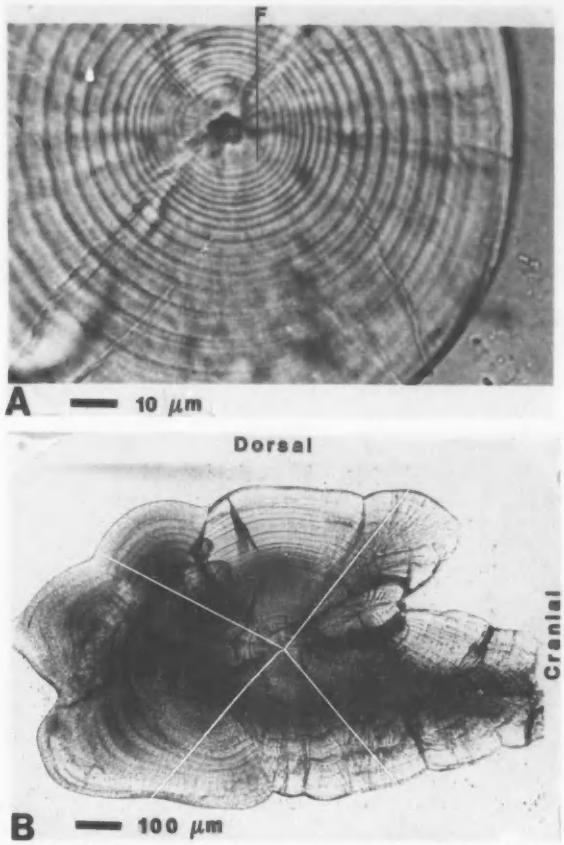

FIG. 2. Otoliths were ground on one side on the sagittal plane relative to the fish: (A) Photograph of sagitta of larval (16 mm) Atlantic mackerel showing central region. Including the first F daily growth ring, 18 completed daily rings were identified. (B) Photograph of sagitta of juvenile (84 mm) Atlantic mackerel; the age was estimated at 48 d. Standard readings fields were inside triangles with apex centered on core, in the dorso-ventral direction.

were obtained whether irregular and diffuse rings were included in the counts or not. Although 21 rings may be observed on Fig. 2A, only the 18 sharp ones were enumerated since the rings identified as daily in the validation experiment presented this characteristic sharpness. Only with the direct validation experiment was it possible to determine that the sharp discontinuous zones were daily, and that the diffuse discontinuous zones were not to be included in the counts for the estimation of age. When counting the number of sharp discontinuous zones from the primordium to the edge of the otolith to estimate age, repeatable estimates were obtained by the two readers (as in Brothers et al. 1976). The high fraction (45%) of otoliths extracted from fish from the validation experiment, but not showing evidence of marking by the calcein, may be attributed to the duration of immersion in the fluorochrome. The fish were maintained in the calcein bath for a period of 2 h, which may have been too brief; for example, Tsukamoto et al. (1989) marked the otoliths of juvenile fish by keeping them for 24 h in the fluorescent substance they used.

The total number of sharp discontinuous zones enumerated on the otolith, below and above the validation size range, was assumed to be equal to as many days of age of the fish since hatching. For fish below the size range over which the validation study was carried out, i.e. for fish smaller than 93 mm, this assumption was partly supported by the results of Migoya (1989) in her study of the growth of larval mackerel. Migoya (1989) hatched mackerel eggs and validated increment formation on the otoliths of larvae at various temperatures. She reported that the ring formed by the first discontinuous zone had a mean radius of 8.1 μm when the fish were raised at 18°C. In our study, the growth discontinuity considered as the first one on the otoliths was usually identified within 7 to 10 μm from the primordium (mean radius: 8.2 μm, SD:0.7 μm; *n*:30). Further, Migoya (1989) indicated that the first of those discontinuous zones would be deposited on the day of, or following, hatching. For these reasons, no extra numbers were added to the actual ring counts, contrary to the procedure of Kendall and Gordon (1981). For fish above the size range over which the validation experiment was carried out, i.e. for fish larger than 170 mm, the correspondence between days of age and ring count was justified by the continuity of the growth equation when passing from inside to outside the size range of the validation.

Lastly, the mean length of wild juvenile mackerel and those in captivity for the validation experiment proved to be similar at corresponding dates (D'Amours and Landry 1989). This suggests that the daily rings on otoliths of the wild fish would be of similar size to those on the otoliths of the captive fish. When ageing the wild fish, the size of the rings was expected to be similar to those identified as daily on the otoliths of the captive fish. This expectation was used to guide the identification of daily rings on the otolith of wild fish.

The conditions in which the fish were maintained during our validation experiment were not varied to test for the effect of various factors on the frequency of ring deposition. The water was pumped directly from the ocean, and the temperature experienced by the detained fish was the same as that experienced by the fish in the wild. The light hours in the laboratory were fewer than in the wild, but consistently so. The main difference in environmental conditions between detained and wild fish was probably the diet. The detained fish were fed about 24% wet body weight/d in three equal portions. Such a high ration was intended to ensure growth, and not affect the frequency of deposition of daily rings. Since the experimental fish were captured as juveniles, it was assumed that the frequency of deposition of the rings was already set, and that differences in environmental conditions in the laboratory would not affect this frequency. As discussed by Campana and Neilson (1982), frequency of ring deposition is not likely to be affected in pre-conditioned young fish exposed to unnatural light or temperature stimuli.

Fitted Growth Curve of Juvenile Mackerel

The one-cycle growth curve fitted to the data provided a generally good description of the length-at-age, although it tended to underestimate size-at-age at its higher end. This discrepancy for fish older than 80 d could be interpreted as the result of a possible underestimation of the age of the larger fish. As noted earlier, the short axis of the otolith was used to estimate the age. According to Wild and Foreman (1980), the counts of growth rings on the short axis of the otolith could underestimate

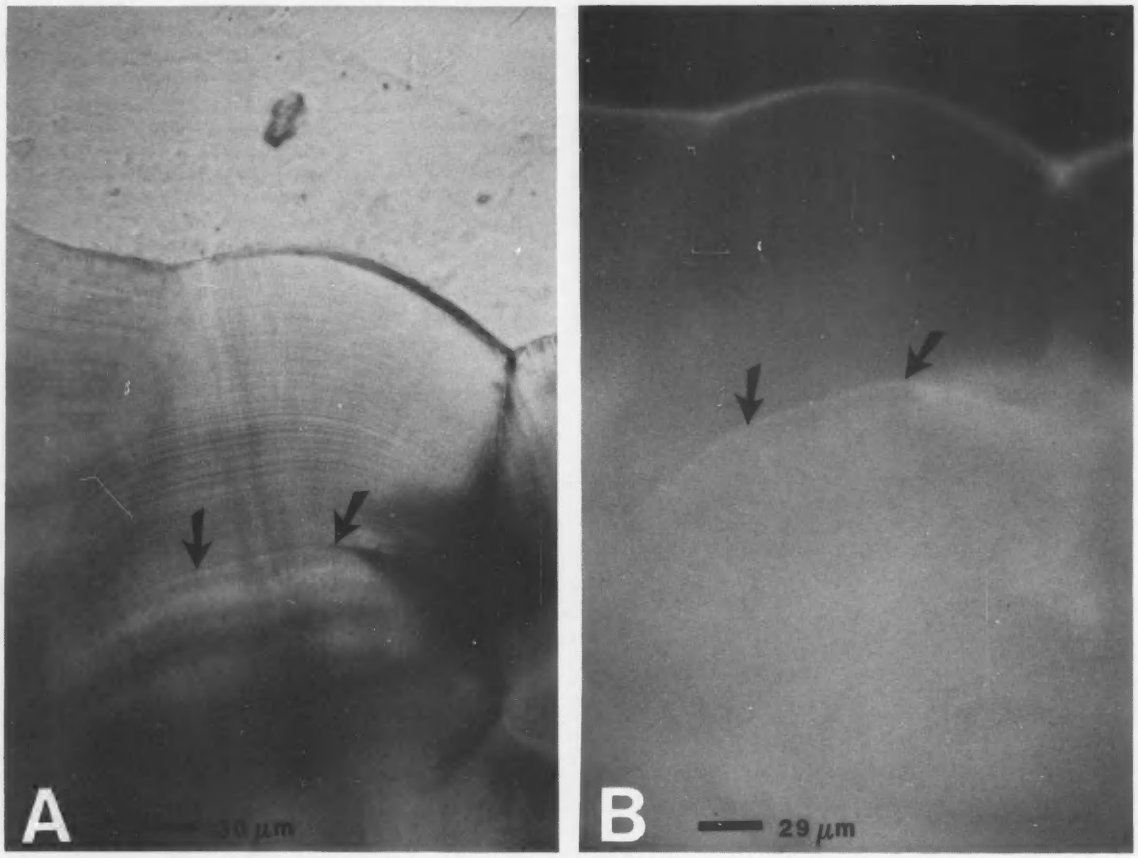

FIG. 3. (A) Photograph of sagitta of juvenile Atlantic mackerel; the otolith was extracted 24 d after the fish had been immersed in calcein for 2 h. A stress check (arrows) was associated with the immersion in calcein. (B) Same preparation as in (A), under reflected U.V. lighting: a fluorescent band (arrows) coincided with the stress check; 25 daily growth rings were identified after the fluorescent band. Note the autofluorescent band on the outer edge of the otolith.

the age of the fish. In future investigations on the growth of juvenile mackerel, the long axis of the otolith should be considered for ageing the fish. This discrepancy at the higher end of the growth curve could also result from the pooling of length-at-age data from fish captured in different years. All the fish younger than 80 d were caught in 1988, while those older had been caught between 1977 and 1980. Although pooling the length-at-age data from different years could affect the growth parameters because of interannual variability in growth rates, this pooling was required to fulfill the objective of this study to describe the growth of juvenile mackerel over the whole range in size during their first growing season, albeit in a composite fashion.

For fish younger than 80 d, the adequacy of the growth model for the data set was evident from the tight correspondence between calculated and observed size-at-age. However, the adequacy of the data set as representative of the whole population of juvenile mackerel may have been affected to an unknown degree by the fish sampling techniques. At the beginning of the sampling period, from July 18 to August 3, young mackerel were collected with a 500 μm plankton net. The largest mackerel caught during this period were 19 mm long. When sampling with the plankton net, the fish above 19 mm (early spawned fish, or faster growing fish) were not available to the net, and the proportion of slower growing fish in the plankton samples may have been higher than in the whole population of 0-yr fish. Less than 4 d later, when juvenile mackerel were collected with a beach seine, the smallest fish captured were 55 mm long. The proportion of early spawned or faster growing fish may have been higher in the beach seine hauls than in the whole population of 0-yr fish. As a result, the growth curve fitted on the data derived from the fish caught with the two sampling techniques could overestimate the growth rate of the fish. The maximum calculated daily growth rate of 2.86 mm/d, could then be slightly inflated.

Another potential source of bias in the calculation of growth rate could result from the fish having different histories of environmental conditions affecting growth. Since the proportion of later spawned juvenile fish in the samples is likely to increase with time, the cumulative effect of environmental conditions on the realized growth could vary and affect the size-at-age relationship. In this study, it was assumed that this bias would be minimal, owing to the relatively narrow hatching window of the fish, and the fast subsequent growth. Lastly, the pooling

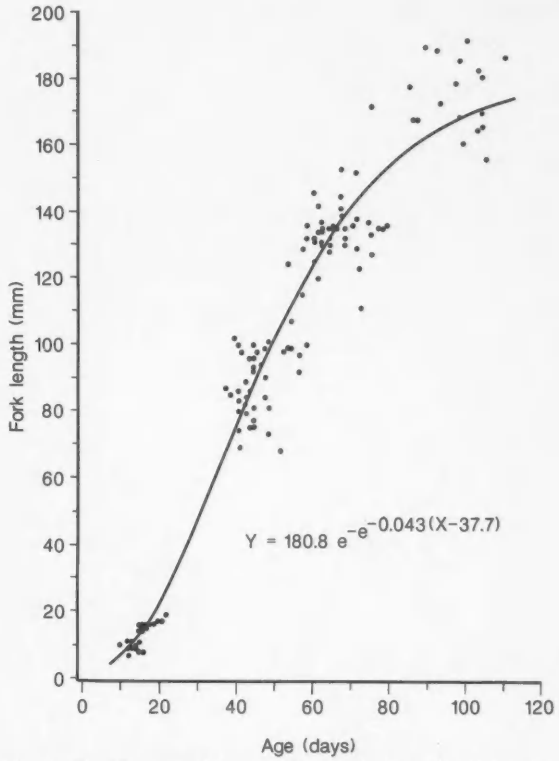

FIG. 4. Growth curve of juvenile (0-yr) Atlantic mackerel from the Gulf of St. Lawrence.

TABLE 1. Parameters of a one-cycle Gompertz curve adjusted on length-at-age with upper and lower 95% C.L. L_∞ = asymptotic fork length (mm), K = growth coefficient, t_o = inflection point (in days of age). Parameters obtained with pooled length-at-age data from juvenile mackerel captured between 1977 and 1980, and in 1988, in the Gulf of St. Lawrence.

		95% Confidence Intervals	
Parameter	Estimate	Lower	Upper
L_∞	180.8	171.3	190.2
K	0.043	0.039	0.047
t_o	37.7	35.9	39.6

of length-at-age from fish caught more than 10 yr apart may have affected the fit of the growth curve to the data. However, it is unlikely that the pooling would invalidate the general growth pattern of the fish during their first growing season, as identified in this study.

Growth of Fish from Northern and Southern Contingents

For the purpose of the discussion, it was assumed that Kendall and Gordon's (1981) results on the growth of young mackerel in the Middle Atlantic Bight were accurate, despite the lack of validation of their ageing technique. Also the growth equation provided by these authors (their Eq. 1) was used directly to compute length-at-age for juvenile mackerel from the south. It was assumed, based on reasons discussed above, that the number of growth increments involved in Kendall and Gordon's (1981) growth equation represented the age of the fish since hatching. Note that Kendal and Gordon (1981) reported standard lengths, while this study reports fork lengths; this difference does not affect the comparison of daily growth increments calculated with the growth equations. Over the age interval (= number of growth increments) 10–20 d, the larval mackerel from the Middle Atlantic Bight would grow on the average by 1.02 mm/d, against 1.38 mm/d in the Gulf of St-Lawrence; over the age interval 20–30 d, the respective mean daily growth rates would be 2.07 and 2.30 mm; over the age interval 30–40 d, their mean daily growth rates would be 2.69 and 2.79 mm. That the young mackerel from the northern contingent would grow faster early in their first growing season would be consistent with Sette's (1950) conclusions, although the sampling bias discussed above would have to be fully addressed before any final conclusion is made. If indeed a difference in growth rate existed between juvenile fish from the two contingents, a difference in the deposition pattern on their respective otoliths should be observed. Such a difference in the microstructure of the otoliths could permit an identification of the contingent of origin of the fish.

Conclusions

The microstructure of the otolith of juvenile mackerel, when properly validated, is a reliable record of age, and allows describing the growth of the fish as a function of absolute age. Secondly, the fish from the northern contingent could grow faster than those from the southern contingent early in their first growing season. However, the effect of sampling selectivity on the computation of growth rates will have to be evaluated before firm conclusions on the existence of a difference in growth patterns between fish from the northern and southern contingents may be reached. This eventual difference could provide the basis for identifying the contingent of origin of adult fish by analysing the microstructure of the initial segment of their otoliths. Lastly, the fast growth rate of juvenile mackerel could be interpreted as an adaptation to grow through a vulnerable size range as quickly as possible. If so, juvenile mackerel would be an ideal species for testing various growth related hypotheses concerning the survivorship of young fishes.

Acknowledgments

We thank Drs. S. Campana, D. Gascon, and M. Castonguay, who made helpful comments on an early version of the manuscript, and two reviewers whose comments considerably improved the manuscript. We also thank the Director and Staff of the Aquarium and Marine Center in Shippegan, for providing us with quality service and equipment.

References

BEAMISH, R. J., AND G. A. MCFARLANE. 1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112: 735–743.

BERRIEN, P. L. 1978. Eggs and larvae of *Scomber scombrus* and *Scomber japonicus* in continental shelf waters between Massachusetts and Florida. Fish. Bull. U.S. 76: 95–115.

BOX, G. E., W. G. HUNTER, AND J. S HUNTER. 1978. Statistics for experimenters. Wiley Series in Probability and Statistics. John Wiley and Sons, New York, NY. 653 p.

BROTHERS, E. B., C. P. MATHEWS, AND R. LASKER. 1976. Daily growth increments in otoliths from larval and adult fishes. Fish. Bull. U.S. 74: 1–8.

CAMPANA, S. E., AND J. D. NEILSON. 1982. Daily growth increments in otoliths of starry flounder (*Platichthys stellatus*) and the influence of some envi-

ronmental variables in their production. Can. J. Fish. Aquat. Sci. 39: 937–942.

1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42: 1014–1032.

D'AMOURS, D., AND J. LANDRY. 1989. Capture and husbandry of juvenile (0-year) Atlantic mackerel (*Scomber scombrus*). Can. Tech. Rep. Fish. Aquat. Sci. 1679: vi + 11 p.

KENDALL, A. W., AND D. GORDON. 1981. Growth rate of Atlantic mackerel larvae in the Middle Atlantic Bight. Rapp. P.-v. Réun. Cons. int. Explor. Mer 178: 337–341.

LAMBERT, T. C., AND D. M. Ware. 1984. Reproductive strategies of demersal and pelagic spawning fish. Can. J. Fish. Aquat. Sci. 41: 1565–1569.

MACKAY, K. T. 1979. Synopsis of biological data of the northern population of Atlantic mackerel (*Scomber scombrus*). Fish. Mar. Serv. Tech. Rep. 885: vi + 26 p.

MIGOYA, C. M. 1989. Étude de la croissance des larves de maquereau bleu de l'Atlantique, (*Scomber scombrus*), à partir de la technique de lecture des otolithes. M.Sc. thesis, Université du Québec à Rimouski, Juin 1989. 60 p.

MORSE, W. W. 1980. Spawning and fecundity of Atlantic mackerel, *Scomber scombrus*, in the Middle Atlantic Bight. Fish. Bull.78: 103–108.

RICKER, W. E. 1973. Growth rates and models, p. 677–743. *In* W. S. Hoar, D. J. Randall, and J. R. Brett [ed.] Fish Physiology Vol. VIII. Academic Press, New York, NY.

SAS INSTITUTE INC. 1985. SAS User's Guide: Statistics, Version 5 ed. Cary, N.C., 956 p.

SETTE, O. E. 1943. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part 1. Early life history. Fish. Bull. 50: 149–237.

1950. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part 2. Migrations and habits. Fish. Bull. 51: 251–358.

SUMMERFELT, R. C., AND G. E. HALL [ED.] 1986. Age and growth of fish. Iowa State University Press, Ames, IA. 544 p.

TSUKAMOTO, K., H. KUWADA, J. HIROKAWA, M. OYA, S. SEKIYA, H. FUJIMOTO, AND K. IMAIZUMI. 1989. Size-dependent mortality of red sea bream, *Pagrus major*, juveniles released with fluorescent otolith-tags in News Bay, Japan. J. Fish. Biol. 35 (Supplement A): 59–69.

WARE, D. M. 1977. Spawning time and egg size of Atlantic mackerel, *Scomber scombrus*, in relation to the plankton. J. Fish. Res. Board Can. 34: 2308–2315.

WARE, D. M., AND T. C. LAMBERT. 1985. Early life history of Atlantic mackerel (*Scomber scombrus*) in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 42: 577–592.

WILD, A., AND T. J. FOREMAN. 1980. The relationship between otolith increments and time for yellowfin and skipjack tunas marked with tetracycline. Inter-Am. Trop. Tuna Comm. Bull. 17: 507–560.

WILSON, C. A., D. W. BECKMAN, AND J. M. DEAN. 1987. Calcein as a fluorescent marker of otoliths of larval and juvenile fish. Trans. Am. Fish. Soc. 116: 668–670.

How Reliable are Growth Back-Calculations Based on Otoliths?

Steven E. Campana

Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2 Canada

Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? Can. J. Fish. Aquat. Sci. 47: 2219–2227.

Growth back-calculations from otoliths assume that the relationship between fish and otolith length is linear through time. The final (or observed) individual fish-otolith ratios are then combined to prepare a fish-otolith regression for the population, upon which the subsequent back-calculations are based. However, recent studies have demonstrated that the fish:otolith size ratio varies systematically with somatic growth rate, resulting in relatively large otoliths in slow-growing fish. Such a growth effect will result in a fitted fish-otolith regression which differs significantly from that of the mean of the individual fish-otolith slopes. Fraser–Lee growth back-calculations made from such a regression consistently underestimate previous lengths at age. The bias may explain the apparent ubiquity of Lee's phenomenon. Back-calculation bias was eliminated through use of an algorithm defining individual fish-otolith trajectories and a biologically determined, rather than a statistically estimated, intercept. Adaptations of the biological intercept back-calculations procedure accurately predicted previous lengths in the presence of both stochastic error and time-varying growth rates. When used to reevaluate some published back-calculations, the biological intercept procedure resulted in more accurate values than those previously estimated, and reduced or eliminated the presence of Lee's phenomenon.

Le calcul inverse de la croissance à partir des otolithes suppose que la relation entre la longueur des otolithes et le poisson est linéaire dans le temps. Les ratios finals (ou observés) otolithes-poisson sont donc combinés en vue de l'obtention d'une régression otolithes-poisson pour la population, à partir de laquelle les calculs inverses ultérieurs se fondent. Cependant, des études récentes ont montré que le ratio poisson-dimensions des otolithes varie systématiquement en fonction du taux de croissance somatique, ce qui correspond à des otolithes relativement gros chez les poissons à croissance lente. Une telle situation conduira à une régression poisson-otolithes particulière qui diffère sensiblement de celle de la moyenne des courbes poisson-otolithes. Les calculs inverses de croissance de Fraser–Lee établis à partir de cette régression sous-évaluent constamment les mesures antérieures de longueur selon l'âge. Le facteur de distorsion peut expliquer l'omniprésence apparente du phénomène de Lee. Le facteur de distorsion des calculs inverses a été supprimé par l'emploi d'un algorithme définissant les circuits otolithes-poisson et un intercept déterminé biologiquement plutôt qu'évalué statistiquement. Les adaptations de la méthode de calcul inverse de recoupement biologique ont permis de prévoir avec exactitude les longueurs antérieures compte tenu de l'erreur stochastique et de taux de croissance variant avec le temps. Utilisée pour réévaluer des calculs inverses rendus publics, la méthode de recoupement biologique a donné des valeurs plus justes que les valeurs estimatives antérieures et a réduit ou éliminé le phénomène de Lee.

Received January 2, 1990
Accepted May 10, 1990
(JA423)

Reçu le 2 janvier 1990
Accepté le 10 mai 1990

Calculation of true growth rate, as opposed to the population growth rate (Ricker 1975), requires data on individual growth trajectories through a period of time if biases associated with gear selectivity and size-selective mortality are to be avoided. Growth back-calculations from periodic features in bony structures, such as scales or otoliths, serve as a commonly-used proxy for sequential observations of size-at-age for individual fish. Of the two underlying assumptions of growth back-calculations, that dealing with constancy in feature periodicity has been dealt with elsewhere, both on the annular (Beamish and McFarland 1983; Casselman 1987) and the daily level (Campana and Neilson 1985). The second assumption, that the distance between consecutive features is proportional to fish growth, has seldom been adequately tested, but has generally been justified on the basis of empirical correlations between otolith/scale size and fish size. While such correlations certainly indicate a general correspondence between the growth rates of the structure and fish, they by no means demonstrate that the correspondence applies on an individual basis or at a detailed level. Such a correspondence would require a constant relationship between otolith/scale growth and fish growth, or at the very least, random error around such a relationship.

Virtually all growth back-calculation procedures are based on proportionality between fish length and some measure of otolith (or scale) size (Carlander 1981). The regression method estimates fish length (L) at some previous age (a) through insertion of the measured size of the otolith (O) at age a into a fish length-otolith length regression derived from samples of the population,

$$(1) \quad L_a = b\,O_a + d$$

where b and d are the slope and intercept of the regression, respectively. Since this procedure assumes no deviation of individual fish and otolith measurements from the overall regression, it has generally been applied when mean back-calculated lengths, rather than individual values, are of importance. In contrast, the more widely-used Lee (or Fraser–Lee) procedure (Carlander 1981) assumes that any deviation of an individual

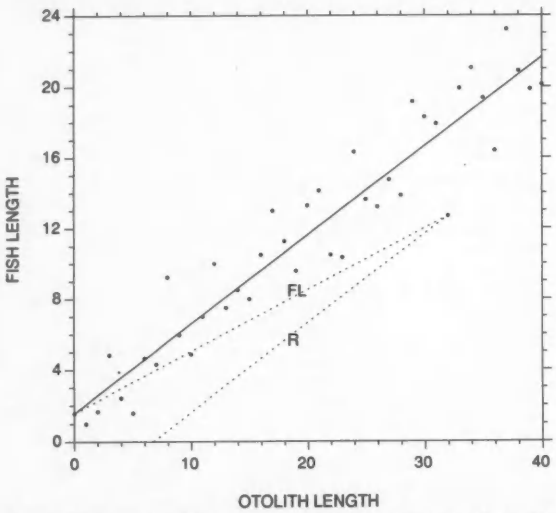

FIG. 1. An example of the regression of fish length on otolith length that is required for the Fraser–Lee and regression growth back-calculation procedures. Regression-based (R) back-calculations from individual fish assume no deviation from the overall regression, while Fraser–Lee (FL) back-calculations assume that individual fish-otolith deviations are maintained proportionally throughout the back-calculation. Both procedures result in mean back-calculated lengths which are equal to the overall fitted regression (solid line).

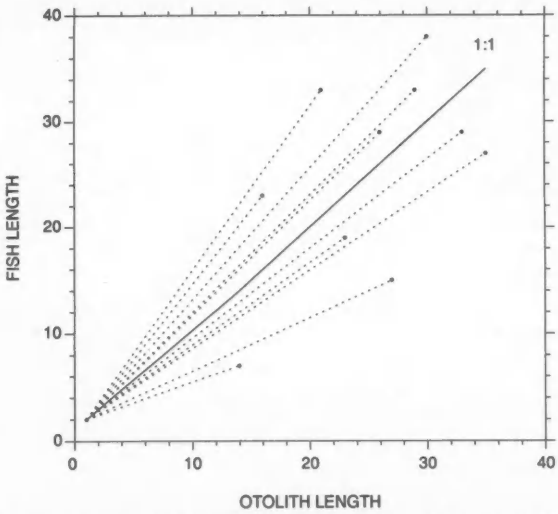

FIG. 2. Conceptual model depicting the trajectories of individual fish lengths relative to otolith lengths. Individual trajectories are not necessarily linear, but on average their slopes are distributed around some mean (1:1) value. Since this is considered to be the traditional (null) model, trajectory slopes are independent of growth rate. The regression predicting fish length from otolith length in the population would normally be calculated using the endpoints of the trajectories, which in this case, would coincide with the mean of the individual slopes.

measurement from the overall fish-otolith regression will be observed proportionally at back-calculated lengths, as in

$$(2) \quad L_a = d + (L_c - d)\, O_c^{-1}\, O_a$$

where L_c and O_c are the fish length and otolith size at capture, respectively. While Eq. 2 does not incorporate the regression slope directly, the value of the regression intercept is, of course, influenced by the slope. Indeed, the regression and Fraser–Lee procedures differ algebraically only in that the latter is intercept-corrected. As a result, the two procedures produce identical mean back-calculated lengths, although back-calculations at the individual level may differ (Fig. 1). Both the regression and the Fraser–Lee procedures are sensitive to age- and sample-dependent variations in the intercept of the fish–otolith length relationship that is employed. Consequently, more sophisticated linear models have been developed to deal with intercept variability (Bartlett et al. 1984; Weisberg 1986; Smith 1987). However, all of the procedures carry the assumption that the fish–otolith length relationship does not vary in a systematic fashion with growth rate, and further, that the regression parameters can be accurately estimated from random samples of the population.

Recent studies of short-term otolith growth have demonstrated that the otolith–fish length relationship can vary systematically with the growth rate of the fish (Mosegaard et al. 1988; Reznick et al. 1989; Secor and Dean 1989a). These studies confirm earlier reports in which otoliths from slow-growing adult fish were consistently larger and heavier than those of fast-growing fish of the same size (Templeman and Squires 1956; Krivobok and Shatunovskiy 1976; Boehlert 1985). The first objective of the current study was to examine the implications of a relationship between growth rate and the otolith-fish size regression: is there any influence on the fish-otolith regression parameters and/or the accuracy of back-calculated values? Also, is there any relationship with the apparent ubiquity of Lee's phenomenon (Lee 1912; Ricker 1969), whereby back-calculated lengths from otoliths of old fish are smaller than the mean lengths actually observed in the population? The second objective is to present a back-calculation procedure with a number of advantages over traditional techniques, including greater accuracy. And the final objective is to use this procedure to re-evaluate some of the conclusions drawn in previous studies which applied back-calculation techniques. While most of the emphasis in this paper will be directed towards growth back-calculations based on daily growth increments, a number of the conclusions will also apply to those based on scale and otolith annuli.

Implications of a Growth Rate Effect upon the Otolith–Fish Length Relationship

Within a population of fish, the trajectories describing individual fish growth relative to otolith growth can be envisioned as having radiated from some common origin (Fig. 2). Positions along the trajectory (which is not necessarily linear) correspond to the size of fish and otolith at a previous time. Smale and Taylor (1987) suggested that an analogous distribution of fish-scale slopes around the mean slope was caused by independent growth rate constants for scale and fish, which covaried in response to some common, controlling mechanism. This suggestion was consistent with the observed increase in the variance of scale size with fish length. A similar process appears plausible for the fish–otolith relationship (Campana and Neilson 1985; Mosegaard et al. 1988). In any event, the concept of individual fish-otolith growth trajectories is well documented (Marshall and Parker 1982; Rosenberg and Haugen 1982; Wil-

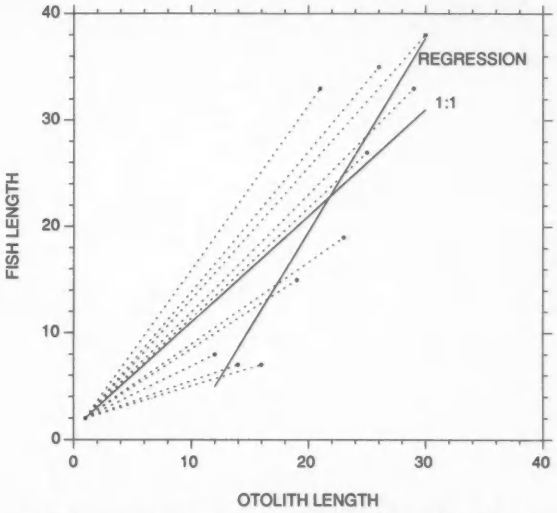

FIG. 3. Conceptual model depicting individual fish-otolith trajectories when faster-growing fish are characterized by smaller otoliths than slower-growing fish of the same size. As in Fig. 2, individual trajectories are distributed around the mean (1:1) slope. However, the trajectories of fast and slow growing fish tend to be segregated above and below the mean, respectively. As a result, the fish-otolith regression calculated on the basis of the trajectory endpoints (e.g. the sampled population) is biased: the calculated slope is too large and the intercept too small.

son and Larkin 1982; Secor and Dean 1989a, b). In Fig. 2, the endpoints of each growth trajectory, corresponding to the lengths of the fish and otolith at the time of sampling, would be used to calculate the overall fish-otolith regression upon which the subsequent back-calculations would be based. In this case, and in all instances where the slopes of the trajectories are independent of growth rate, the fitted regression line coincides with the mean (1:1) fish-otolith trajectory.

Direction of Bias Induced by Growth Rate Effects

The phenomenon whereby slow-growing fish have larger otoliths than fast-growing fish of the same size is widespread, both at the microstructural (Taubert and Coble 1977; Marshall and Parker 1982; McGurk 1984; Miller and Storck 1984; Neilson et al. 1985; Penney and Evans 1985; Post and Prankevicius 1987; West and Larkin 1987; Mosegaard et al. 1988; Reznick et al. 1989; Secor and Dean 1989a, b; Wright et al. 1990; but see Tzeng and Yu 1988) and the annular (Templeman and Squires 1956; Krivobok and Shatunovskiy 1976; Boehlert 1985) level. The experiments of Rice et al. (1985) also show evidence of the phenomenon (when their otolith-fish regressions are constrained through the point corresponding to size-at-hatch).

When incorporated into the conceptual model of Fig. 2, the growth rate phenomenon would act to segregate the slow and fast growing fish on either side of the mean fish-otolith growth trajectory (Fig. 3). Thus the slower growing fish, characterized by shorter trajectories, would tend to occur below the mean trajectory, while the faster growing fish with longer trajectories would occur above the mean. While the slope of the mean trajectory would probably remain unaffected by any such segregation, the effect upon the calculated regression, which is based upon the trajectory endpoints, is striking (Fig. 3). Irrespective of the magnitude of the growth effect that is postulated, the slope of the regression will always be greater, and the intercept less, than that of the mean trajectory. On the basis of Fig. 2 and 3, the bias in the fitted regression would appear to be an inescapable product of a population characterized by a range of growth rates in which faster growing fish have relatively small otoliths, and vice versa. Since a fish-otolith regression based upon the trajectory endpoints is the normal predecessor of any back-calculation procedure, bias can also be expected of any subsequent back-calculations, such that back-calculated lengths are smaller than observed at the time. These conclusions are interesting from two perspectives. First, they suggest that bias in back-calculated lengths can arise even when predictions are made within the data range of the regression, and despite random sampling of the population. Indeed, if the growth effect on the fish to otolith length ratio is as universal as now seems likely, these results indicate that back-calculation bias should be an expected result of otolith-based back-calculations. Secondly, the consistent underestimation of fish length at earlier ages would appear to provide a plausible explanation for Lee's phenomenon. The apparent ubiquity of Lee's phenomenon has always been somewhat of an enigma, and although size-selective mortality may well contribute to the phenomenon (Ricker 1969), it now appears that growth effects can produce a similar result in the absence of size-selective mortality.

Magnitude of Bias Induced by Growth Rate Effects

Bias in the fish-otolith regression will result in detectable back-calculation error only if the bias is of sufficient magnitude. To assess the relative importance of the bias on back-calculation accuracy, the conceptual model of Fig. 2 was used to simulate the somatic and otolith growth of a cohort of fish. The objective was to determine the net fish–otolith relationship in a cohort comprised of fish of varying growth rates, where the slopes of the individual fish-otolith trajectories were influenced by their growth rates (e.g. Fig. 3). The cohort ($n = 100$) was assumed to have hatched on a single day with initial fish and otolith lengths equal to zero. Use of non-zero intercepts (e.g. otoliths present in fish at time of hatch) has no influence on the results of this model. To mimic the expected natural variability in growth rates, otolith growth rates were normally distributed among fish. Individual growth rates were kept constant through the 50-d period of growth; a model with time-varying growth rates is presented later. The algorithm for the model is:

(i) $G \sim N(0.5, 0.1)$ across i fish
(ii) $O_{it} = G_i \times t$
(iii) $\text{Ratio}_i = (1 - R) + R \times (G_i/\bar{G})$
(iv) $L_{it} = O_{it} \times k \times \text{Ratio}_i$

where G_i is the daily growth rate of the ith otolith, $\bar{G}$ is the mean otolith growth rate, O_{it} is the length of the otolith in the ith fish after t days of growth, Ratio_i is the daily fish length increment to otolith length increment ratio in the ith fish, k (set to 1.0 in this model) is the conversion constant between fish and otolith growth rates, R is the magnitude of the growth rate effect on the daily fish to otolith ratio, and L_{it} is the length of the ith fish after t days. Note that this formulation is deterministic in that the daily otolith and fish growth rates are directly linked; a model with stochastic error around both growth rate constants is presented later. The calculation of the daily fish to

otolith ratio (Ratio) results in a value of 1.0 for fish growing at the mean growth rate or for cohorts where the growth effect is absent ($R=0$). However, when $R>0$, faster-growing fish would produce relatively small otoliths, and vice versa. As the value of R is increased, so does the discrepancy in otolith size for fish of a given length but different growth rates. In this model, Ratio is constant within a fish, since individual growth rates have been assumed to be constant through time. The influence of time-varying growth rates (and Ratios) is considered in a later model. Note also that the Ratio calculation is presented as a function of otolith growth, rather than somatic growth, due to the fact that otolith size is the independent variable in the subsequent regression. However, rearrangement of the Ratio calculation as a function of somatic growth does not affect the conclusions which follow. While there are few available data upon which to base the assumption of a linear effect of growth rate on the fish to otolith ratio, use of a nonlinear effect does not alter the implications of the model results.

Irrespective of the period of growth, the simulated cohort was characterized by a normal distribution of both fish lengths and otolith lengths, each centred around a similar mean. Preparation of the standard fish-otolith regression, based upon the individual fish and otolith lengths at the end of the growth period, resulted in populations with a slope of 1.0 and a zero intercept whenever the growth effect (R) was set to zero. As the magnitude of the growth effect (R) was increased, there were proportional increases and decreases in the values of the fitted regression slopes and intercepts, respectively. Back-calculated lengths based on these regression parameters became increasingly biased (too small) as the value of the growth effect was increased above 0. In the presence of a strong growth effect ($R=1.0$), the slope of the fish-otolith regression was more than doubled, indicating the potential for substantial back-calculation error. The degree of bias was virtually independent of the coefficient of variation (CV) in growth rates present in the simulated population. Similar results were observed when the stochastic error ($e_i \sim N(0, 1)$) expected of a natural population was introduced around the fish to otolith ratio calculations (line (iii) in model algorithm), as in:

$$\text{Ratio}_i = ((1 - R) + R \times (G_i/\bar{G}) + e_i$$

Estimation of the Value of the Growth Effect (R)

The model results indicate that back-calculation bias will result from any non-zero value of the effect of growth rate (R) upon the daily fish to otolith ratio. However, small values of R may not produce detectable bias in natural situations. To estimate the magnitude of R that exists under natural conditions, independent estimates were derived from published data, through regression of the slope of the fish-on-otolith regression on growth rate,

$$(3) \quad S = a' + R\,G$$

where S is the observed slope of the fish length-on-otolith regression, G is the observed absolute linear rate of growth in fish length, and a' and R (the growth effect) are parameters to be estimated. To facilitate the comparison of the regression results among studies, the growth and slope data entered into each regression were first standardized with respect to their means.

Three studies provided sufficient data to estimate the magnitude of the growth effect (R). In the first, Reznick et al. (1989) reported only a 4% difference in the adjusted mean length of guppy (*Poecilia reticulata*) otoliths from two treatments where growth rate varied by a factor of 2.3. While statistically correct, their analysis of covariance of the relationship between otolith and fish length assumed homogeneity of slopes and different intercepts between the treatments. Since the environmental and genetic histories of all of the experimental fish were similar and carefully controlled, it is more reasonable to assume that the treatments shared a common intercept, but had different slopes reflecting the different growth regimes. On this basis, and using the regressions presented in their figure 1, the treatment slopes of fish length on otolith length differed by 24%, resulting in a normalized fish-otolith slope which increased at 27% of the normalized rate of increase of growth. A slightly higher estimate of the influence of growth was obtained from four growth treatments in a study of sockeye salmon (*Oncorhynchus nerka*) otoliths by Marshall and Parker (1982) ($R = 0.42$; $n = 4$; $P = 0.16$). However, the most robust estimate of the growth effect was calculated from inverse regressions (which replaced otolith length by fish length as the dependent variable) of the striped bass (*Morone saxatilis*) data of Secor and Dean (1989a). The resulting estimate indicated that the normalized slopes of the fish-on-otolith regression increased at 42% of the rate of increase of growth rate ($n = 12$; $P < 0.0001$). Given the differences among the species and experimental designs used in these three studies, a value of 0.3 would appear to be a conservative estimate of the relative influence of the growth effect on the fish to otolith ratio.

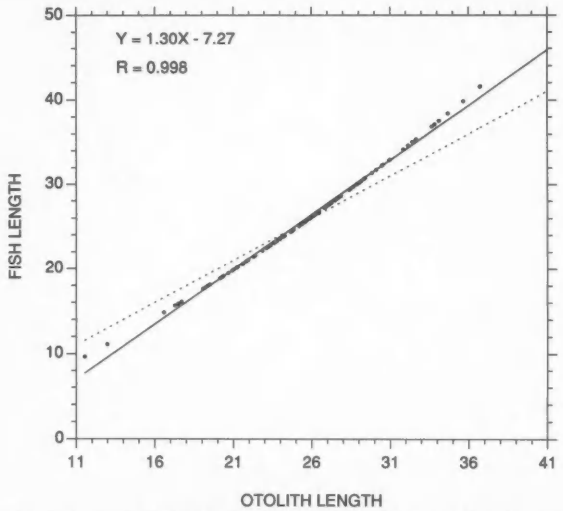

FIG. 4. Example of the distribution of individual fish-otolith trajectory endpoints from a cohort when faster-growing fish are characterized by smaller otoliths than slower-growing fish of the same size (sensu Fig. 3). The value of the growth rate effect ($R=0.3$) was estimated from published data, and was entered into the model deterministically. The fitted regression (solid line) is the normal predecessor to growth back-calculation, yet it is significantly different ($P<0.01$) from that of the mean fish-otolith trajectory (dotted line; slope = 1.0; intercept = 0).

Incorporation of $R = 0.3$ as the magnitude of the growth effect in the fish-otolith simulation model demonstrated the amount of bias that would result in the subsequent back-calculation. After a 50-d period of growth, preparation of the

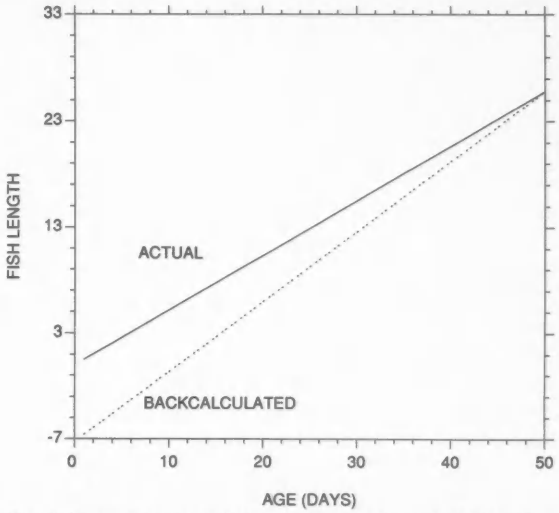

FIG. 5. Fraser–Lee growth back-calculations of mean length based on the fish-otolith trajectory endpoints in Fig. 4. The back-calculated lengths-at-age underestimated the actual lengths in this and all other simulations where growth rate influenced the slope of the fish-otolith trajectories. The actual and back-calculated lengths are significantly different ($P<0.01$).

standard fish-otolith regression from the "sampled" cohort revealed what would normally be interpreted as a strong and reliable correlation, despite the presence of a barely detectable pattern in the residuals (Fig. 4). However, the slope and the intercept of the regression were significantly ($P < 0.01$) larger and smaller, respectively, than those of the mean of the individual trajectories. A similar result was observed in simulations where a normal error distribution was placed around the growth–ratio relationship. The variance in the latter simulation, which is almost certainly present in the natural environment, effectively obscured any evidence of a pattern in the residuals.

Growth back-calculations of the simulated cohort were made on an individual basis with both the Fraser–Lee and regression methods. Both procedures produced mean back-calculated values which were significantly smaller ($P < 0.01$) than the actual lengths at the time (Fig. 5). Both the absolute and percent deviation from actual values increased as the origin of the regression was approached.

Variations of the fish-otolith simulation model demonstrated that back-calculation bias persisted in the presence of both multiple cohorts and size-selective sampling (mimicking gear selectivity whereby the smallest and/or largest fish in the population were unavailable to the gear). However, the magnitude of the bias was greatly reduced in instances where newly hatched cohorts were present at the time of sampling, presumably through their high leverage on the fish-otolith regression (by forcing the slope through, or near to, the true origin). The latter implies that, in general, back-calculations from daily growth increments in otoliths from juvenile fish collections may be more susceptible to back-calculation bias than those from larval fish. Simulations of multiple annual cohorts, where growth rate declined with age, resulted in significantly different fish-otolith regression parameters among ages, thus providing a plausible explanation for the relative success of back-calculation procedures where age is entered as a variable (Bartlett et al. 1984; Miller and Storck 1984). However, the age-specific regression parameters were also biased, due to growth rate variability within each age-group.

The relative influence of the fish-otolith regression intercept on the accuracy of growth back-calculations has been presented by several workers (Carlander 1982; Smale and Taylor 1987). The use of species-specific intercepts has been recommended as one means of removing statistical uncertainty due to sampling errors (Carlander 1982). Other researchers have ensured the consistency of their estimated intercept values with independent estimates (Crecco and Savoy 1985; West and Larkin 1987) or have applied procedures which explicitly recognized the possibility of age-specific intercepts (Bartlett et al. 1984; Weisberg 1986). However, to my knowledge, this is the first demonstration that population-wide back-calculation bias can result from other than sampling error, and irrespective of the statistical analysis that is applied.

Minimization of Growth Back-Calculation Bias due to Growth Rate Effects

Use of a Biological Intercept

Under the assumption of proportionality between fish and otolith lengths, the Fraser–Lee back-calculation procedure effectively calculates a separate slope for each fish, using the intercept of the population-based regression as a starting point. By definition, this starting point corresponds to the fish length at which otolith length equals zero, rather than the origin of the fish-otolith trajectories presented in Fig. 2. Independent of the growth effect described earlier, the Fraser–Lee method cannot be used to accurately reconstruct the individual fish-otolith trajectories of Fig. 2; there is no single Y-intercept value (corresponding to a regression intercept) which can be used as an origin for all of the individual trajectories. However, given an appropriate value for the regression intercept, the Fraser–Lee method will correctly predict mean back-calculated lengths, even in the presence of a growth effect. This property suggests that the Fraser–Lee method is an appropriate starting point for the development of a new back-calculation procedure, requiring only a better means of defining the intercept to be complete.

In all of the experiments where growth has been manipulated or monitored, it has been the slope of the fish-otolith trajectories which has varied, rather than the intercept (Marshall and Parker 1982; Rosenberg and Haugen 1982; Rice et al. 1985; Secor and Dean 1989a, 1989b). Indeed, given a common pool from which experimental fish are drawn, all should, by definition, share a common intercept. The common intercept in this case is biologically, not statistically based, and corresponds to the size of the otolith and fish at the beginning of the experiment. Generally, I would define the biological intercept of a fish-otolith trajectory as the fish and otolith length corresponding to the initiation of proportionality between fish and otolith growth. In many species, this point would occur at the time of hatch.

The use of biological intercepts in a modification of the Fraser–Lee back-calculation procedure eliminates the errors and bias that were described in previous sections. When tested in the cohort simulation model, the equation

$$(4)\quad L_a = L_c + (O_a - O_c)(L_c - L_o)(O_c - O_o)^{-1}$$

accurately predicted previous lengths, both at the individual and population level, back to the biological intercept (fish = L_o, otolith = O_o). Equation (4) simply describes the

line connecting an individual fish-otolith coordinate at the time of sampling to that at the biological intercept (sensu Fig. 2 or Fig. 3). In other words, the calculation is intercept-corrected along both X and Y axes. Although the equation was not presented, Smale and Taylor (1987) made a similar recommendation with respect to back-calculations from scale annuli. In contrast, Carlander (1982) concluded that biologically based intercepts might not be reliable, and suggested that back-calculation error be minimized through use of standardized regression intercepts. While the standardization of regression intercepts would almost certainly reduce back-calculation error due to sampling variability, it would not necessarily eliminate bias due to statistical estimation of the standardized intercept. Of course, bias would be minimal if the regression included enough observations near the origin to lever the regression through the biological intercept.

The biological intercept back-calculation procedure has a number of desirable features which recommend its use. Firstly, through incorporation of intercept corrections along both fish and otolith axes, the procedure fits the widely accepted conceptual model of Fig. 2 more closely than any other back-calculation model. Secondly, it is insensitive to bias induced by variable growth rates in the population, although not to growth rate variations through time. Thirdly, the size of the fish and otolith at hatch (or at the time of initiation of a linear fish–otolith relationship) can be precisely measured, and differs little among individuals. Fourthly, back-calculation accuracy is relatively insensitive to normal variation around the intercept value, largely because of the small values involved. Most importantly, use of this procedure does not rely on parameter estimation from a sample of the population. Indeed, back-calculations can be made from an individual fish in the absence of any other fish from the population, other than those used to verify proportionality between otolith and fish growth after the biological intercept. In many cases, the biological intercept could be determined by simple measurements of otolith and fish size in newly-hatched larvae in the laboratory. As for disadvantages of the procedure, I was not able to identify any that were not also associated with other back-calculation techniques. Foremost of these were those linked to nonlinear effects, as discussed in the following section.

Time-Varying Growth Rates

All of the available back-calculation procedures, including Eq. 4, assume linearity in individual fish-otolith trajectories throughout the lifetime of the fish. However, it is unlikely that an individual fish would maintain a constant growth rate through time; thus, the effect of growth rate on the fish–otolith relationship would be expected to introduce curvature into individual fish-otolith trajectories. Here, I will use an enhancement of the fish-otolith simulation model to assess the effect of such nonlinearities on back-calculation accuracy, and suggest improvements to Eq. 4 so as to reduce the influence of time-varying growth rates.

Using the same 100-fish cohort and growth effect ($R = 0.3$) described earlier, individual fish-otolith growth trajectories were modeled over a 150-d period, with a normally distributed error around both the growth rate ($G \sim N(0.5, 0.1)$ and the fish to otolith ratio (Ratio $\sim N(1.0, 0.1)$. Each fish experienced a "life history transition," characterized by a fourfold increase in growth rate (from 0.25 to 1.0 units/d), at an arbitrary size of 20 units (corresponding to a mean age of 80 d). The variance

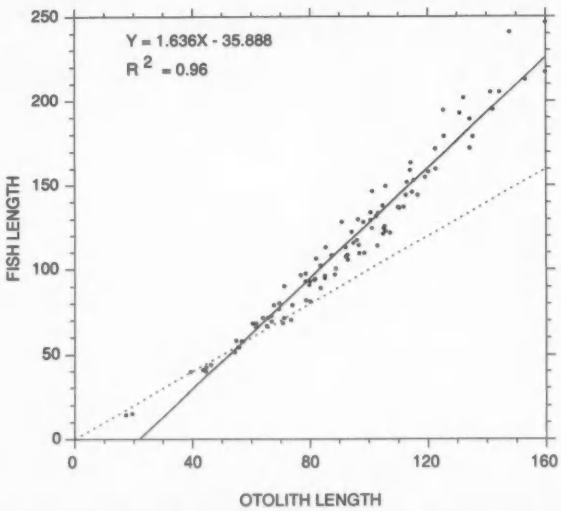

FIG. 6. A fish-otolith regression fitted to simulated data from a 150-d-old cohort which experienced individual life history transitions, characterized by a four-fold increase in growth rate, at a length of 20. The slope and intercept of the regression (solid line) are significantly different from the mean fish-otolith trajectory slope and intercept of 1.0 and 0, respectively (dotted line). The growth rate effect on individual trajectory slopes was set at 0.3, but included a normal error distribution around the mean daily fish:otolith ratio (Ratio $\sim N(1, 0.1)$).

in growth rates among fish implies that individuals would undergo the transition at different ages. Since the point of transition was designed to simulate natural conditions, it was calculated as a function of size, not age (Policansky 1982; Chambers and Leggett 1987). The extreme case of an abrupt transition was intentionally imposed to maximize the nonlinearity in the individual fish-otolith trajectories. Back-calculations were made on the basis of the "observed" population at the end of the 150-d period.

The characteristics of the net fish-otolith regression (Fig. 6) were similar to those observed in Fig. 4, with the exception that a pattern in the residuals could not be discerned. The slope and intercept were significantly larger and smaller ($P < 0.01$) than 1.0 and 0, respectively. Back-calculations made with both the Fraser–Lee and the biological intercept methods differed from actual values (Fig. 7), although the size of the deviation was much more pronounced and consistently negative in the former. Back-calculations made with the Fraser–Lee method differed from actual values by 159% at the 63-d age corresponding to the lower limit of the regression data in Fig. 6. The percentage deviation increased as the origin was approached. In contrast, the deviation of the biological intercept method never exceeded 42% and was negligible at the time of hatch. Similar results were observed in other simulations where the growth rate transition was a function of age (simulating a sudden environmental effect), and where the fish–otolith relationship was deterministic rather than stochastic.

In principle, it is possible to use a series of daily growth increment widths to compensate for back-calculation curvature produced by time-varying growth rates. Since the width of a daily increment is a measure of daily otolith growth rate, compensation for the growth rate effect, on a day-to-day basis,

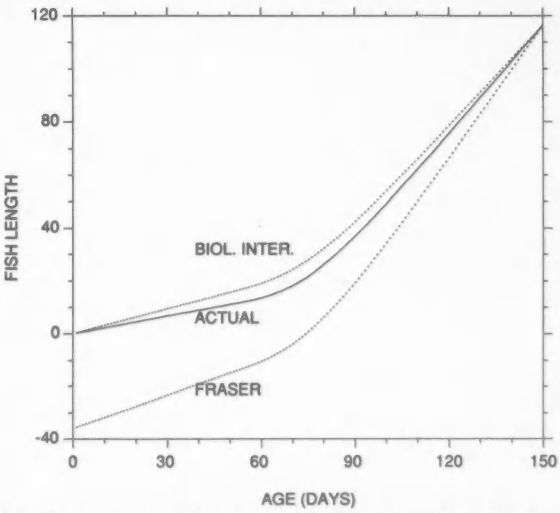

FIG. 7. A comparison of actual mean lengths-at-age with those back-calculated from the observations in Fig. 6, in which individual growth rates varied through time. Back-calculations made with the linear form of the biological intercept procedure (Biol. Inter.) (Eq. 4) were more accurate than those made with the Fraser–Lee method, but both deviated from actual values. Mean back-calculated values made with Eq. (7) were the same as the actual values, although individual back-calculations differed.

should restore proportionality to the increment width series, as in:

$$(5)\quad L_t = W_t \times k \times \text{Ratio}_t$$

In other words, the daily growth in length at age t (L_t) is related to the width of the corresponding increment (W_t) by a conversion constant (k) and the fish–length growth ratio on that day (Ratio_t). If the magnitude of the growth effect (R) is known, and substituting line (iii) of the model algorithm for Ratio, the calculation becomes

$$(6)\quad L_t = W_t \times k \times ((1 - R) + R \times (W_t \times \overline{W}^{-1}))$$

with $\overline{W}$ defined as the mean daily increment width (or otolith growth rate) across all fish and ages. Length back-calculation is then the sum of daily back-calculated fish length increments, as in:

$$(7)\quad L_a = L_o + \sum_{t=1}^{a} (k \times W_t \times ((1 - R) + R \times (W_t \times \overline{W}^{-1})))$$

where L_o is the size of the fish at the origin of the linear fish-otolith trajectory (e.g. biological intercept), and $t = 1$ corresponds to the first day after L_o. The requirement for a continuous series of increment widths, from the time of hatch (or biological intercept) to the point of back-calculation, makes this formulation somewhat more restrictive than Eq. 4. It also does not account for stochastic error around the mean daily fish to otolith ratio. As a result, back-calculations made with Eq. 7 are analogous to those made with the regression method, whereby mean back-calculated lengths are more accurate than those at the individual level.

Despite the above caveats, Eq. (7) accurately predicted mean back-calculated lengths in the cohort simulation which incorporated time-varying growth rates (Fig. 7). The maximum absolute deviation from actual lengths for individual fish was only 8%, making the calculation considerably more accurate than either the biological intercept or the Fraser–Lee methods at both the individual and the mean level. However, Eq. (7) should be used with caution, given its potential sensitivity to the untested assumptions concerning the error distribution and age independence of the growth rate effect. The formulation of line (iii) in the model algorithm would also have to be developed with more rigor than was done here, since $\bar{G}$ could be expected to vary ontogenetically or under different environmental conditions.

In practice, estimates of the magnitude of the growth effect can be made without an experimental framework. Given the value of the biological intercept, each fish in a sample could serve as an independent observation in the regression of Eq. 3. Each fish-otolith length trajectory could be used to calculate the dependent variable (the slope of the fish-otolith regression), while examination of the otolith microstructure would provide the age estimate required for calculation of the independent variable (growth rate). Of course, the sample would have to be selected from an environment in which time-varying growth rates were minimized.

It is important to note that Eq. 7 cannot be used to compensate for all sources of nonlinear fish-otolith trajectories. At the daily level, autocorrelated increment widths (Gutiérrez and Morales–Nin 1986; Bradford and Geen 1987) would be expected to reduce the accuracy of detailed growth back-calculations. In principle, a time series model could be developed to account for the autocorrelation, but such would require considerably more research. Deviations from a linear fish to otolith trajectory at a frequency greater than that of the periodic feature being used would also introduce error. Examples of such deviations would include seasonal disruptions of the fish to otolith ratio (Reay 1972; Casselman 1987) and their effects upon back-calculations made from annuli. For this reason, and given inter-annual variations in the timing of annulus formation, Eq. 7 is more likely to be of value when applied at the daily level.

Implications for Previously Published Studies

In many situations, the difference between growth back-calculations made with traditional methods and those made with the biological intercept procedure will be relatively small. Under conditions where the statistical and biological intercepts of the fish-otolith regression are collinear, mean back-calculated lengths will be identical, although individual values may differ. More substantial differences can be expected under the following circumstances: (1) when there are differences between the biological and statistical intercepts, (2) when different fish-otolith regressions are used for different populations, and (3) when the same fish-otolith regression is used for different populations of dissimilar growth rates. Using these criteria, I have re-examined a number of published studies, both as an independent test of the validity of the biological intercept back-calculation procedure, and to determine the implications of its use, if any, on the authors' conclusions.

Differences in the collinearity between the biological and statistical intercept can be a major source of back-calculation error. Both Crecco and Savoy (1985) and West and Larkin (1987) gave careful attention to this problem, and demonstrated consistency between estimated and observed intercept values before their application. However, in a study of redfish

(*Sebastes* spp) larvae (Penney and Evans 1985), there was a demonstrable difference (evident in the residuals) between the observed and predicted fish to otolith ratios at larval extrusion, which may have had implications for the conclusions that were reached concerning early larval growth.

A more substantial difference between statistical and biological intercepts was apparent in a study of back-calculated growth in sand lance (*Ammodytes dubius*) (Scott 1973). The statistically estimated fish length at which otoliths were formed (e.g. the statistical intercept) was 2.4 cm (Scott 1973), a value which was six times larger than the actual value (0.4 cm; Scott and Scott 1988). The discrepancy was probably the product of the fish-otolith regression bias discussed earlier. As noted by Scott (1973), regression-based back-calculations of mean length underestimated the observed mean lengths-at-age by up to 33%. However, when I repeated the back-calculations using the biological intercept procedure (Eq. 4), virtually all evidence of Lee's phenomenon disappeared: all back-calculated values fell to within 6% of observed values.

Additional support for the validity of the biological intercept procedure was obtained in a re-evaluation of back-calculated growth in multiple year-classes of juvenile chinook salmon (*Oncorhynchus tshawytscha*) (Neilson et al. 1985). While it had no effect on their conclusions, Neilson et al's (1985) back-calculated sizes at entry into the estuary by the 1979 cohort underestimated observed values. The underestimation was due to the relatively large calculated value for the fish-otolith regression slope, which in turn was almost certainly due to the regression bias produced by the rapid growth rate of that cohort. Application of the biological intercept procedure to their data produced mean back-calculated lengths that were 12% closer to observed values than were the corresponding back-calculations based on the Fraser–Lee method. There were no significant differences between the biological and statistical intercepts, nor between the corresponding back-calculations, for the remaining year-classes.

Use of a single fish–otolith relationship to back-calculate the growth of two samples with different growth rates appears to be the cause of an interesting anomaly in the study of Wilson and Larkin (1982). After a careful analysis of the sequential growth of a single cohort, Wilson and Larkin (1982) reported a good correspondence between back-calculated and observed fish weights, but noted a parabolic distribution of unknown origin in the residuals. Re-examination of their data indicated that the growth rate declined by over a factor of 2 in the second half of their experiment. Based on my simulations, their residual pattern matched that expected of two disparate growth rates and the corresponding change in the fish to otolith ratio through the course of the experiment. In an unrelated study, Post and Prankevicius (1987) also used a single fish-otolith regression to back-calculate the growth of different samples. After combining inshore and offshore collections that were characterized by significantly different growth rates, the authors concluded that size-selective mortality was the cause of differences between observed and back-calculated lengths in one of the lakes. Application of the biological intercept procedure to their digitized data reduced, although it did not eliminate, the size of the discrepancy. In the second lake, where size-selection was not apparent, the revised back-calculated lengths matched those that were observed. In a third study, Beacham (1981) could find no evidence of density-dependent growth after applying a single fish–otolith relationship to samples from a sequence of year-classes. Since density-dependent growth would be expected to manifest itself as reduced growth of abundant year-classes, and given the growth rate effect on the fish to otolith ratio, it is doubtful that he could have detected density-dependent growth with the regression method that he applied. Further analysis would be required to confirm or reject his hypothesis.

In summary, re-analysis of a variety of independent studies with the biological intercept back-calculation procedure confirmed its effectiveness in estimating previous lengths at age at least as accurately, and generally more so, than was possible with the Fraser–Lee method. In most instances, the revised back-calculation reduced the magnitude of length underestimation and eliminated the need to invoke Lee's phenomenon. The assumptions of the biological intercept procedure were better met than those of the Fraser–Lee method in all studies; in some cases, use of the former produced results which were inconsistent with the conclusions reached through use of the latter.

Conclusions

The realization that the fish to otolith ratio is influenced by somatic growth rate necessitates some rethinking of traditional means of growth back-calculation. This is particularly true given that the effect of growth variability is to induce a bias into the net fish–otolith relationship, upon which all traditional back-calculation procedures are based. The bias is evident at both the daily and the annular level. Fortunately, this information can be used to recommend an alternative, and in many ways easier, back-calculation algorithm. While based upon a different rationale, some of the features of the biological intercept technique are common to those associated with Carlander's (1982) standard intercept formulation. Additional advantages of the former include increased resistance to bias associated with growth rate variations, increased back-calculation accuracy at the individual and mean level, ease of determination of the intercept value, and in particular, insensitivity to the sampling error and truncation effects that can be so influential in other back-calculation procedures (Carlander 1981; Bartlett et al. 1984; Smale and Taylor 1987). The algorithm is applicable at both the daily and the annular level. All techniques can produce error when back-calculations are made outside of the sample range or when curvilinear fish–otolith relationships are left untransformed. However, a further advantage of the biological intercept algorithm is that fish-otolith proportionality can be confirmed in other, independent samples, and need not be repeated in each back-calculation study. Nonlinear fish–otolith relationships formed as a consequence of time-varying growth rates require special handling (e.g. Eq. 7), and in many populations, will probably result in the introduction of some back-calculation error.

The finding that Lee's phenomenon can result from mathematical bias induced by the effect of growth on the fish to otolith ratio is noteworthy, given the number of reports of its existence (Halliday 1969; Scott 1973; Bagenal and Tesch 1978; Neilson et al. 1985; Radtke et al. 1985; Morales–Nin 1988). The fact that similar bias may exist in back-calculations based on scale annuli suggests that further research on the fish–scale relationship may be required (Carlander 1981; Smale and Taylor 1987).

While the influence of growth rate on the fish to otolith ratio has been both quantified and applied in this study, little is known of the underlying mechanisms. It is unlikely that growth rate is the only variable which can influence relative otolith size

(Mosegaard et al. 1988; Secor and Dean 1989a, 1989b; Wright et al. 1990). Identification of other influential variables would not affect the conclusions drawn here concerning statistical bias in the fish-otolith regression, or the overall benefits of the biological intercept procedure. However, it could alter, and presumably improve, our perception of individual fish-otolith trajectories in the presence of conditions promoting time-varying growth rates. It could also improve our ability to back-calculate previous lengths under such conditions.

Acknowledgements

The technical assistance of J. Hamel is greatly appreciated. J. D. Neilson, D. H. Secor, S. J. Smith, and an anonymous referee offered many helpful comments on an earlier version of the manuscript. N. J. Wilimovsky introduced me to some of the key concepts discussed in this paper, and to him I express my gratitude.

References

BAGENAL, T. B., AND F. W. TESCH. 1978. Age and growth, Chap. 5 p. 101–136. *In* T. Bagenal [ed.] Methods for Assessment of Fish Production in Fresh Waters. Blackwell Scientific Publ. London, UK.

BARTLETT, J. R., P. F. RANDERSON, R. WILLIAMS, AND D. M. ELLIS. 1984. The use of analysis of covariance in the back-calculation of growth in fish. J. Fish. Biol. 24: 201–213.

BEACHAM, T. D. 1981. Variability in growth during the first 3 years of life of cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. Can. J. Zool. 59: 614–620.

BEAMISH, R. J., AND G. A. MCFARLANE. 1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112: 735–743.

BOEHLERT, G. W. 1985. Using objective criteria and multiple regression models for age determination in fishes. Fish. Bull. 83: 103–117.

BRADFORD, M. J., AND G. H. GEEN. 1987. Size and growth of juvenile chinook salmon back-calculated from otolith growth increments, p. 453–461. *In* R. C. Summerfelt and G. E. Hall [eds.] Age and Growth of Fish. Iowa State University Press, Ames, IA.

CAMPANA, S. E., AND J. D. NEILSON. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42: 1014–1032.

CARLANDER, K. D. 1981. Caution on the use of the regression method of back-calculating lengths from scale measurements. Fisheries 6: 2–4.

1982. Standard intercepts for calculating lengths from scale measurements for some centrarchid and percid fishes. Trans. Am. Fish. Soc. 111: 332–336.

CASSELMAN, J. M. 1987. Determination of age and growth, p. 209–242. *In* A. H. Weatherley and H. S. Gill [ed.] The Biology of Fish Growth. Chap. 7 Academic Press, New York, NY.

CHAMBERS, R. C., AND W. C. LEGGETT. 1987. Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. Can. J. Fish. Aquat. Sci. 44: 1936–1947.

CRECCO, V. A., AND T. F. SAVOY. 1985. Effects of biotic and abiotic factors on growth and relative survival of young American shad, *Alosa sapidissima*, in the Connecticut River. Can. J. Fish. Aquat. Sci. 42: 1640–1648.

GUTIÉRREZ, E., AND B. MORALES–NIN. 1986. Time series analysis of daily growth in *Dicentrarchus labrax* L. otoliths. J. Exp. Mar. Biol. Ecol. 103: 163–179.

HALLIDAY, R. G. 1969. Population parameters of *Argentina sphyraena* (Isospondyli) from west of Britain. J. Mar. Biol. Assoc. U.K. 49: 407–431.

KRIVOBOK, M. N., AND M. I. SHATUNOVSKIY. 1976. Influence of gonad maturation on otolith weight increase in the Baltic cod, *Gadus morhua callarias*. J. Ichthyol. 16: 408–414.

LEE, R. M. 1912. An investigation into the methods of growth determination in fishes. Cons. Perm. Int. Explor. Mer Publ. de Circonstance 63: 35 p.

MARSHALL, S. L., AND S. S. PARKER. 1982. Pattern identification in the microstructure of sockeye salmon (*Oncorhynchus nerka*) otoliths. Can. J. Fish. Aquat. Sci. 39: 542–547.

MCGURK, M. D. 1984. Ring deposition in the otoliths of larval Pacific herring, *Clupea harengus pallasi*. Fish. Bull. 82: 113–120.

MILLER, S. J., AND T. STORCK. 1984. Temporal spawning distribution of largemouth bass and young-of-the-year growth, determined from daily otolith rings. Trans. Am. Fish. Soc. 113: 571–578.

MORALES–NIN, B. 1988. Age determination in a tropical fish, *Lethrinus nebulosus* (Forskal, 1775) (Teleosteii: Lethrinidae) by means of otolith interpretation. Invest. Pesq. 52: 237–244.

MOSEGAARD, H., H. SVEDANG, AND K. TABERMAN. 1988. Uncoupling of somatic and otolith growth rates in Arctic char (*Salvelinus alpinus*) as an effect of differences in temperature response. Can. J. Fish. Aquat. Sci. 45: 1514–1524.

NEILSON, J. D., G. H. GEEN, AND D. BOTTOM. 1985. Estuarine growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) as inferred from otolith microstructure. Can. J. Fish. Aquat. Sci. 42: 899–908.

PENNEY, R. W., AND G. T. EVANS. 1985. Growth histories of larval redfish (*Sebastes* spp.) on an offshore Atlantic fishing bank determined by otolith increment analysis. Can. J. Fish. Aquat. Sci. 42: 1452–1464.

POLICANSKY, D. 1982. Influence of age, size, and temperature on metamorphosis in the starry flounder, *Platichthys stellatus*. Can. J. Fish. Aquat. Sci. 39: 514–517.

POST, J. R., AND A. B. PRANKEVICIUS. 1987. Size-selective mortality in young-of-the-year yellow perch: evidence from otolith microstructure. Can. J. Fish. Aquat. Sci. 44: 1840–1847.

RADTKE, R. L., M. L. FINE, AND J. BELL. 1985. Somatic and otolith growth in the oyster toadfish (*Opsanus tau* L.). J. Exp. Mar. Biol. Ecol. 90: 259–275.

REAY, P. J. 1972. The seasonal pattern of otolith growth and its application to back-calculation studies in *Ammodytes tobianus*. J. Cons. Int. Explor. Mer 34: 485–504.

REZNICK, D., E. LINDBECK, AND H. BRYGA. 1989. Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). Can. J. Fish. Aquat. Sci. 46: 108–112.

RICE, J. A., L. B. CROWDER, AND F. P. BINKOWSKI. 1985. Evaluating otolith analysis for bloater *Coregonus hoyi*: do otoliths ring true? Trans. Am. Fish. Soc. 114: 532–539.

RICKER, W. E. 1969. Effects of size-selective mortality and sampling bias on estimates of growth, mortality, production, and yield. J. Fish. Res. Board Can. 26: 479–541.

1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

ROSENBERG, A. A., AND A. S. HAUGEN. 1982. Individual growth and size-selective mortality of larval turbot (*Scophthalmus maximus*) reared in enclosures. Mar. Biol. 72: 73–77.

SCOTT, J. S. 1973. Otolith structure and growth in northern sand lance, *Ammodytes dubius*, from the Scotian Shelf. ICNAF Res. Bull. 10: 107–115.

SCOTT, W. B., AND M. G. SCOTT. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. 219: 731 p.

SECOR, D. H., AND J. M. DEAN. 1989a. Somatic growth effects on the otolith–fish size relationship in young pond-reared striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 46: 113–121.

SECOR, D. H., J. M. DEAN, AND R. B. BALDEVARONA. 1989b. Comparison of otolith growth and somatic growth in larval and juvenile fishes based on otolith length/fish length relationships. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 191: 431–438.

SMALE, M. A., AND W. W. TAYLOR. 1987. Sources of back-calculation error in estimating growth of lake whitefish, p. 189–202. In R. C. Summerfelt and G. E. Hall [ed.] Age and Growth of Fish. Iowa State University Press. Ames, IA.

SMITH, P. J. 1987. Maximum likelihood estimation of the distribution of length at age. Biometrics 43: 601–616.

TAUBERT, B. D., AND D. W. COBLE. 1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. J. Fish. Res. Board Can. 34: 332–340.

TEMPLEMAN, W., AND H. J. SQUIRES. 1956. Relationship of otolith lengths and weights in the haddock *Melanogrammus aeglefinus* (L.) to the rate of growth of the fish. J. Fish. Res. Board Can. 13: 467–487.

TZENG, W.-N., AND S.-Y. YU. 1988. Daily growth increments in otoliths of milkfish, *Chanos chanos* (Forsskal), larvae. J. Fish. Biol. 32: 495–504.

WEISBERG, S. 1986. A linear model approach to backcalculation of fish length. J. Am. Stat. Assoc. 81: 922–929.

WEST, C. J., AND P. A. LARKIN. 1987. Evidence for size-selective mortality of juvenile sockeye salmon (*Oncorhynchus nerka*) in Babine Lake, British Columbia. Can. J. Fish. Aquat. Sci. 44: 712–721.

WILSON, K. H., AND P. A. LARKIN. 1982. Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry. Can. J. Fish. Aquat. Sci. 39: 1335–1339.

WRIGHT, P. J., N. B. METCALFE, AND J. E. THORPE. 1990. Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L: evidence against coupling. J. Fish. Biol. 36: 241–249.

Effect of Temperature on the Chronic Toxicity of Arsenate to Rainbow Trout (*Oncorhynchus mykiss*)

S. M. McGeachy[1] and D. G. Dixon[2]

Department of Biology, University of Waterloo, Waterloo, Ont. N2L 3G1 Canada

McGeachy, S. M., and D. G. Dixon. 1990. Effect of temperature on the chronic toxicity of arsenate to rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 47: 2228–2234.

The effects of water temperature (5 or 15°C) on the toxicity of arsenate to rainbow trout were examined over an 11-wk period. The fish were exposed to nominal arsenate concentrations set as fixed proportions of the 144-h LC50s for arsenate at their respective temperature (5°C: 0, 1.5, 18, and 36 mg·L^{-1}; 15°C: 0, 1.5, 9, and 18 mg·L^{-1}). Arsenate toxicity was assessed in terms of mortality, total arsenic concentration, wet weight, condition factor, liver and muscle glycogen levels, hepato- and splenosomatic indices, and histopathology. Contrary to the previously reported relationship between acute toxicity and temperature, trout were more tolerant of chronic exposure to arsenate at 15°C than at 5°C. While the high-exposure concentration (5°C, 36 mg·L^{-1}; 15°C, 18 mg·L^{-1}) fish at both temperatures attained the same internal arsenic concentration (2 to 3 μg·g^{-1}), up to 50% of those fish tested at 5°C died. The whole-body arsenic concentrations in moribund trout were found to vary between 4 and 6 μg·g^{-1}, suggesting that a critical arsenic body-concentration is reached before death or toxicant insult occur.

Les effets de la température de l'eau (5 ou 15°C) sur la toxicité de l'arséniate pour la truite arc-en-ciel ont été examinés pendant une période de 11 sem. Les poissons ont été exposés à des concentrations nominales d'arséniate établies selon des proportions fixes de la CL50 — 144 h aux températures indiquées (5°C : 0, 1.5, 18 et 36 mg·L^{-1}; 15°C : 0, 1.5, 9 et 18 mg·L^{-1}). La toxicité de l'arséniate a été évaluée d'après la mortalité, la concentration totale d'arsenic, le poids net, le coefficient de condition, les teneurs en glycogène du muscle et du foie, les indices hépato et splenosomatiques et l'histopathologie. Contrairement à la relation signalée précédemment entre la température et la toxicité aiguë, les truites toléraient mieux une exposition chronique à l'arséniate à 15°C qu'à 5°C. Bien que les poissons exposés aux fortes concentrations (5°C, 36 mg·L^{-1}; 15°C, 18 mg·L^{-1}) aux deux températures aient présenté la même concentration interne d'arsenic (2 à 3 μg·g^{-1}), jusqu'à 50 % des poissons soumis aux tests à 5°C sont morts. On a observé que les concentrations d'arsenic dans tout l'organisme des truites moribondes variaient de 4 à 6 μg·g^{-1}, ce qui porte à croire que l'organisme atteint une concentration critique d'arsenic avant la mort ou l'aggression par le produit toxique.

Received September 18, 1989
Accepted May 10, 1990
(JA309)

Reçu le 18 septembre 1989
Accepté le 10 mai 1990

Temperature plays a critical role in establishing the distribution limits, rates of function, and survival of many organisms, including fish (Fry 1971; Hochachka and Somero 1984). Temperature is one of the more extensively studied environmental variables (Cairns et al. 1975). Since fish are poikilotherms, their biochemical and metabolic processes are directly controlled by temperature. Due to temperature's varied effect on metabolism, and metabolism's influence on the uptake, biotransformation, depuration and toxicity of chemicals, no assumptions should be made about the effects of temperature on the toxicity of waterborne contaminants to aquatic organisms (Sprague 1985).

Arsenic can be released into the environment from both anthropogenic (mining, steel production, fossil fuel combustion, insecticide use) and natural (leaching of arsenic rich soils) sources (Ferguson and Gavis 1972; National Research Council of Canada 1978). Although arsenic can exist in numerous inorganic and organic forms in the environment, the pentavalent cation is the most stable inorganic form in aquatic systems (Gulledge and O'Connors 1973). Since the distribution of environmental arsenic contamination encompasses numerous habitats and biomes, modifying factors such as temperature have the potential to significantly alter the toxic response of organisms to arsenic.

Previous work by McGeachy and Dixon (1989) found the acute toxicity of arsenate, but not of arsenite, to be altered by temperature. Rainbow trout (*Oncorhynchus mykiss*) tested at 5°C were more resistant to arsenate than those tested at 15°C. Our previous study also suggested that there is a critical whole-body concentration of arsenic associated with death. Regardless of arsenate exposure concentration, temperature, or duration of exposure, dead trout showed whole-body arsenic concentrations of from 8 to 12 μg·g^{-1}. The 15°C trout reached this critical concentration and died more rapidly than the trout tested at 5°C when both were exposed to the same waterborne arsenate concentration.

The purpose of this study was to investigate the impact of temperature (5 and 15°C) on the chronic toxicity (11 wk) of arsenate to rainbow trout. Effects of chronic exposure to arsenate were assessed in terms of mortality, changes in wet and dry weight, condition factor, glycogen levels in liver and mus-

[1]Present address: New Brunswick Department of Fisheries and Aquaculture, P.O. Box 5001, St. Stephen, New Brunswick, E3L 2X4 Canada.

[2]Author to whom correspondence should be addressed.

cle, hepato- and splenosomatic indices, whole-body arsenic concentration, and the presence or absence of histopathological lesions in gill, gall bladder, liver, kidney, and axial muscle. In light of the reported relationship between arsenic body concentrations and morbidity (McGeachy and Dixon 1989), whole-body arsenic levels were measured in moribund trout throughout the study.

Materials and Methods

Fingerling rainbow trout, with an initial mean weight of 5.7 g, were obtained from the Spring Valley Trout Farm, St. Petersburg, Ontario. After a 1-wk period in the laboratory at 10°C, the fish were randomly distributed to 16 continuous-flow 50-L aquaria (100 per aquarium) where the water temperature was adjusted to either 5 or 15°C by increments of 1°C per day. Once the test temperature had been attained, the fish were subjected to a further 2.5-wk acclimation period prior to the start of the arsenate exposure. Throughout the acclimation period the trout were fed a semi-purified diet at a rate of 1% (5°C) or 2% (15°C) wet weight·d^{-1}. The diet, containing 50% protein, 25% carbohydrate, 15% fish oil, and 10% vitamin premix, was formulated as described by Morito et al. (1986). During arsenate exposure the fish were fed a minimum of four times daily to the amount recommended by Hilton and Slinger (1981). The percent ration fed ranged from 1.7% wet weight·d^{-1} at 5°C to 4.1% wet weight·d^{-1} at 15°C. These levels were to near satiety and were considered optimal for the trout at each body size and holding temperaure. The daily feeding ration, adjusted weekly, was based on the total aquarium biomass for each replicate. All test aquaria were cleaned of faecal material daily.

The ground water used in this study was tested once every 2 wk and had the following mean (SE) chemical characteristics: alkalinity, 279(2) mg·L^{-1} as $CaCO_3$; total hardness, 377(4) mg·L^{-1} as $CaCO_3$; Ca hardness, 245(7) mg·L^{-1} as $CaCO_3$; and pH, 7.93 (0.05). Oxygen levels, measured weekly, were kept at 80–90% saturation via continuous aeration. The photoperiod was 16 h light: 8 h dark, with 0.5 h of gradual dawn and dusk included in the light portion of the cycle. Temperature was measured daily in each tank. The mean (SE) temperatures over the 11-wk exposure period for the two temperature regimes were 5.3 (0.1) and 15.3 (0.1)°C.

The trout were exposed to sodium arsenate ($Na_2HAs0_4 \cdot 7H_20$, standard grade, Mallinckrodt Ltd.) for 11 wk at four concentrations in duplicate. The nominal concentrations used were 0.0, 1.5, 9.0, and 18.0 mg·L^{-1} at 15°C and 0.0, 1.5, 18.0, and 36.0 mg·L^{-1} at 5°C. The exposure levels were selected in accordance with the 144-h LC50s for arsenate at 5 and 15°C (McGeachy and Dixon 1989), with the highest concentration corresponding to 0.30 of the LC50 at each temperature. Water samples were taken daily from each tank, acidified to 1% with analytical grade nitric acid, and stored for determination of arsenic concentration by neutron activation analysis (McGeachy and Dixon 1989). Mean in-tank assayed arsenic concentrations ranged from 85 to 98% of nominal with a mean coefficient of variation of 17.5 (Table 1). All the data in this paper were analyzed in terms of assayed arsenic concentration. The arsenate was delivered into the test aquaria by a modified Mount–Brungs diluter (Lemke et al. 1978) at a flow of 850 mL·min^{-1}·$aquarium^{-1}$. This gave a 95% replacement time of 3 h, within the range recommended by Sprague (1969).

All mortalities were recorded within 24 h of the time of occurrence. On days 7, 14, 28, and 77, subsamples of fish were taken from each replicate for the determination of wet and dry weight and length. Condition factor was calculated as 100 (weight·$length^{-3}$). At the end of the experiment, liver and spleen weights were recorded (± 0.001 g) and hepato- and splenosomatic indices were calculated as 100 (organ weight·$body\ weight^{-1}$).

Four fish were removed from each tank (eight per treatment) on days 1, 3, 7, 14, 28, 63, and 77 for total whole-body arsenic analysis. The fish were killed by over-anaesthetization with tricaine methane sulphonate, rinsed in deionized water for 1–2 min to remove any surface arsenic contamination, weighed, measured, frozen in liquid nitrogen (−196°C) and stored at −20°C pending analysis. Moribund fish were sampled in the same fashion. Prior to arsenic analysis whole fish were freeze-dried to a constant weight using a Virtis lyophilizer (Model 10-100). Whole-body arsenic content was determined by neutron activation, as previously described (McGeachy and Dixon 1989).

TABLE 1. Mortalities of rainbow trout during an 11-wk exposure to arsenate at 5 or 15°C.

Temperature (°C)	Nominal As concentration (mg·L^{-1})	Mean (SE) assayed As concentrate (mg·L^{-1})	Replicate	Initial sample size	Number of mortalities
5	0	0.01 (0.01)	1	95	0
	0	0.01 (0.01)	2	95	0
	1.5	1.36 (0.02)	1	96	0
	1.5	1.42 (0.02)	2	96	0
	18	16.3 (0.08)	1	93	3
	18	16.2 (0.11)	2	96	5
	36	32.9 (0.21)	1	92	25
	36	33.7 (0.14)	2	92	31
15	0	0.01 (0.01)	1	86	0
	0	0.01 (0.01)	2	85	0
	1.5	1.40 (0.10)	1	84	0
	1.5	1.48 (0.10)	2	84	0
	9	8.52 (0.14)	1	83	1
	9	8.20 (0.04)	2	84	1
	18	17.7 (0.11)	1	85	6
	18	18.4 (0.08)	2	85	7

On days 28 and 77, four fish from each tank were killed and samples of liver and muscle were excised, immersed in liquid nitrogen, and stored at −20°C for determination of liver and muscle glycogen by the method of Passoneau and Lauderdale (1974). An additional four fish were taken from each tank and portions of the gill, gall bladder, liver, kidney, and dorsal muscle were fixed in 10% buffered formalin for histological examination. The preserved tissues were embedded in paraffin, sectioned (9 μm) and stained with haematoxylin and eosin using standard methods (Department of Pathology, University of Guelph, Guelph, Ontario).

Data were analysed using a split-plot ANOVA design to test for the effects of time, arsenic concentration, and temperature using standard SAS programs from the library of the Department of Computer Services, University of Waterloo. Multiple comparisons were made by a protected LSD method recommended for split-plot designs (Steele and Torrie 1980). A test for homogeneity was performed on each data set, with any heterogeneous data being log transformed and re-analysed. Since the fish were sampled for liver and muscle glycogen, histology, and arsenic content at different time intervals, the mortality data had to be censored to allow the use of proper statistical procedures (SAS Life-Test). This procedure also determined a corrected percent mortality, since sampling progressively reduced the total number of trout being exposed, fish which may have succumbed to arsenic if they had not been sampled. Mortality only occurred with the medium- and high-exposure groups at each temperature, so statistical comparisons for mortality were limited to those treatments. Due to a significant difference in the variability between tanks and fish, all analysis for arsenic body concentration, wet weight, and dry weight used the mean values from the individual tanks as the sample observations. The data were log-transformed.

Results

Whole-body arsenic concentration could be correlated with arsenate exposure level as early as day 1 at both 5 and 15°C (Fig. 1). Body concentrations in the 5°C high-arsenate treatments peaked by day 7 (2.5–3.0 μg As·g wet weight^{-1}) and showed no statistically significant changes ($P>0.05$) with time during the balance of the exposure (Fig. 1). At both temperatures the body concentrations in low-exposure groups remained at levels similar to the control fish throughout the exposure period. The pattern of arsenic accumulation at 15°C was similar to the pattern at 5°C up to day 63, after which significant decreases ($P<0.05$) in arsenic concentration were apparent in the medium (50.6%) and high (49.9%) exposure groups. It must be emphasized that although the arsenate exposure concentrations for the high-arsenate treatments were 18 and 36 mg·L^{-1} at 5 and 15°C respectively, the fish achieved essentially the same whole-body arsenic concentrations (Fig. 1). This was also true of the medium-exposure treatments at both temperatures.

Fish mortality during this study did not follow any detectable pattern (regression $B = 0$; $P = 0.48$) and occurred throughout the 11-wk experimental period. No deaths were observed in the control and low-arsenate treatment groups at either temperature (Table 1). Of the medium- and high-arsenate treatments at the two temperatures, the replicates of the high-arsenate concentration at 5°C showed the highest levels of mortality (replicate 1, 27%; replicate 2, 34%). Since fish were sampled at different intervals for other parameters, the mortality rates were corrected (45 and 56% respectively) to reflect the progressively

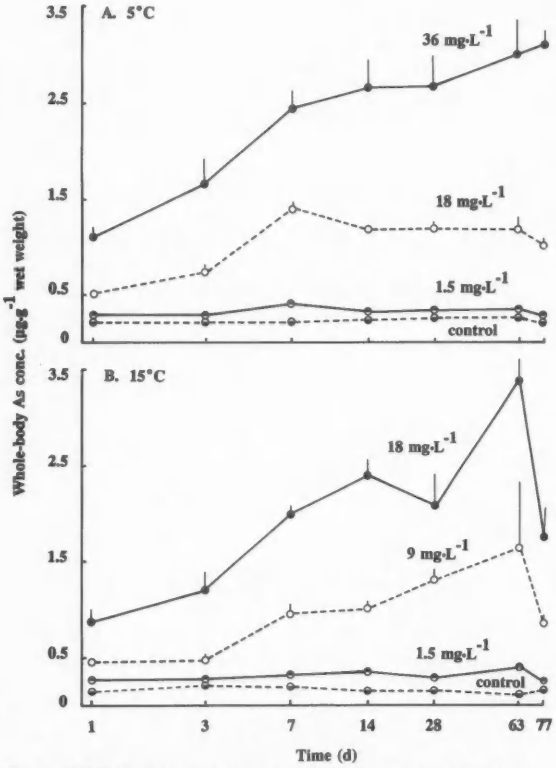

FIG. 1. Whole-body arsenic concentrations shown by rainbow trout at 5(A) or 15(B)°C during a 77-d exposure to control (0.0 mg·L^{-1}; ⊖---⊖), low (1.5 mg·L^{-1}; ⊖——⊖), medium (9 and 18 mg·L^{-1} at 15 and 5°C respectively; ○---○) or high (18 and 36 mg·L^{-1} at 15 and 5°C respectively; ●——●) arsenate concentrations. Each point is given as a mean with the standard error. Where no error bar is given, the standard error falls within the diameter of the point.

decreasing population size during the study. Mortality rates between all replicates within each treatment were not significantly different, so the data were pooled for further analysis. Analysis of the pooled treatments found mortality to be dependent on the waterborne arsenate concentration, relative to the LC50, at each temperature. When mortalities of fish exposed to 18 mg·L^{-1} were compared between the two temperatures, there was no difference ($P=0.0751$, $\chi^2 = 3.168$). However, when comparisons were made with the exposure concentrations expressed as a proportion of the 144-h LC50 (0.30 LC50; 36 mg·L^{-1} at 5°C or 18 mg·L^{-1} at 15°C), more of the low temperature fish died due to arsenic toxicity than the 15°C fish ($P=0.0001$, $\chi^2=29.004$). The same conclusion can be made if mortalities are compared when whole-body arsenic concentrations are the same. As noted above, although whole-body arsenic concentrations were virtually the same in the high-treatment fish at both temperatures, fish held at 5°C showed significantly higher mortality than the 15°C fish. When the data were pooled across temperature, the 5°C fish were once again more sensitive to arsenic than the 15°C fish ($P=0.001$, $\chi^2=26.684$). Mortality at the higher exposure level was significantly greater than at the medium level regardless of the temperature ($P=0.0001$, $\chi^2=59.023$).

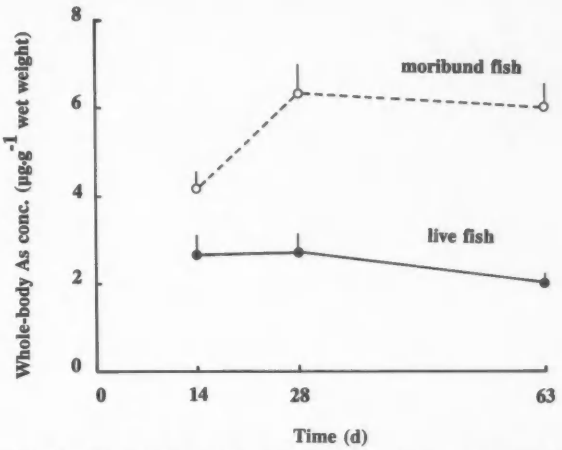

FIG. 2. Whole-body arsenic concentrations shown by moribund (O---O) and live-sampled (●——●) rainbow trout exposed to arsenate at 36 mg·L^{-1} for 77 d at 5°C. Each point is given as a mean with the standard error.

Analysis of whole-body arsenic content in moribund trout was limited to those fish in the high-arsenate, 5°C treatment because of the high incidence of mortality at that concentration. On days 14, 28, and 63, moribund trout showed a significant ($P<0.0001$) elevation in arsenic body concentration relative to live fish sampled at the same time (Fig. 2). The moribund trout had a mean (SE) concentration of 5.36 (0.67) μg·g^{-1}, while the sampled trout had a mean (SE) concentration of 2.03 (0.25) μg·g^{-1}. The relationship between whole-body arsenic concentration and time for moribund trout (Fig. 2) suggested a slight increase in arsenic content between day 14 (4.15 μg·g^{-1}) and day 63 (6.02 μg·g^{-1}). Analysis of variance, however, showed the effect of time to be nonsignificant ($P=0.171$), most probably due to a significant time by sampling procedure interaction ($P=0.033$).

Both temperature and time had the most notable effect on wet weight gain during arsenic exposure (Table 2). The irregular growth pattern exhibited by all of the 5°C treatments was related to a highly significant interaction between time and temperature ($P < 0.0001$). The control, low-, and medium-exposure groups showed a slow but gradual increase in wet weight with no detectable effects of arsenate. Although the highest exposure group showed no appreciable change in wet weight during the first 28 d of exposure, it was not until day 77 that this reduced weight gain became statistically significant ($P < 0.05$). Weight gain at 15°C was, not surprisingly, significantly greater ($P < 0.0001$) than at 5°C. At 15°C the control, low, and medium groups showed significant increases in mean wet weight at all time periods, while the high exposure group showed no changes in wet weight during the first 14 d. At 15°C there was no significant effect of low- and medium-arsenate exposure on wet weight gain. Due to poor growth performance during the first 14 d of exposure, the trout of the 15°C high-arsenate treatment remained significantly lighter than the remaining 15°C treatments after 77 d of exposure. The patterns of change shown by dry weight measurements were essentially the same as those for wet weight at both temperatures.

The overall lack of any appreciable effect on wet and dry weight gain is reflected in the absence of effects on condition factor (Table 3). The condition factor analysis assumed no differences between replicates in a factorial ANOVA design (concentration × temperature). The results suggest a slight temperature by concentration interaction at the medium and high exposure levels and an increased condition factor at 15°C relative to 5°C ($P=0.0412$). A Tukey's multiple comparisons test revealed that the condition factor for the high treatment at 5°C was significantly lower than all groups at both temperatures, except for medium 5°C and control 15°C. There were no marked effects of arsenate on either the hepatosomatic or splenosomatic index (Table 3). Although a large interaction factor obscured any individual dose effects, the pooled 5°C treatments had a higher hepatosomatic index than the 15°C treatments ($P<0.05$). The degree of nonsignificance was stronger with the splenosomatic index for both concentration exposure ($P=0.439$) and temperature ($P=0.208$).

Arsenate exposure level and time significantly affected liver ($P=0.032$) and muscle ($P=0.0039$) glycogen content (Table 4). There were no significant effects of arsenate or temperature on liver glycogen levels on day 28. Only those fish in the 5°C low-arsenate treatment showed a significant change in liver glycogen levels with time; the mean level increased from 29.3 mg·g^{-1} on day 28 to 40.7 mg·g^{-1} on day 77. By day 77 the mean liver glycogen concentrations shown by the fish of the high-arsenate treatments at both temperatures were significantly reduced relative to all other treatments. Due to a highly nonsignificant third order interaction (concentration × temperature × time), multiple comparisons of the muscle data had to be computed with pooled data. This analysis (protected LSD for split-plot) showed that by day 77, the 15°C trout had less

TABLE 2. The wet weights shown by rainbow trout on days 1, 14, 28, and 77 of an 11-wk exposure to arsenate at either 5 or 15°C. Each value is the pooled mean (SE) of two replicates with a total sample size of from 16 to 18.

Temperature (°C)	Nominal exposure concentrations (mg As·L^{-1})	Mean weight (g)			
		Day 1	Day 14	Day 28	Day 77
5	0	8.0 (0.9)	9.4 (0.5)	9.3 (0.5)	15.6 (0.3)
5	1.5	8.2 (0.2)	9.4 (0.6)	10.1 (0.3)	15.0 (0.1)
5	18.0	8.0 (0.1)	10.1 (1.3)	9.8 (0.1)	14.4 (0.6)
5	36.0	8.4 (0.2)	8.9 (0.2)	8.4 (0.6)	10.4 (0.9)
15	0	7.0 (0.1)	9.5 (0.1)	13.9 (0.6)	34.2 (1.9)
15	1.5	7.5 (0.3)	9.3 (0.4)	15.3 (0.6)	40.2 (1.7)
15	9.0	7.1 (0.1)	8.9 (0.5)	12.3 (0.5)	35.0 (0.5)
15	18.0	6.9 (0.6)	7.5 (0.2)	10.5 (0.5)	26.0 (2.6)

TABLE 3. Condition factor (K), hepatosomatic index (HSI) and splenosomatic index (SSI) shown by rainbow trout after 77 d of exposure to arsenate at 5 or 15°C. Each value is the pooled mean (SE) of two replicates.

Temperature (°C)	Nominal exposure concentration (mg As·L^{-1})	K	HSI	SSI
5	0	1.09 (0.01)	1.29 (0.06)	0.12 (0.01)
5	1.5	1.08 (0.02)	1.24 (0.08)	0.10 (0.01)
5	18.0	1.05 (0.02)	1.51 (0.03)	0.15 (0.01)
5	36.0	0.99 (0.02)	1.47 (0.08)	0.20 (0.09)
15	0	1.07 (0.02)	0.94 (0.09)	0.11 (0.02)
15	1.5	1.09 (0.02)	0.95 (0.05)	0.09 (0.01)
15	9.0	1.12 (0.03)	1.32 (0.26)	0.09 (0.01)
15	18.0	1.08 (0.03)	1.12 (0.02)	0.12 (0.02)

TABLE 4. Liver and muscle glycogen concentrations shown by rainbow trout on days 28 and 77 of an 11-wk exposure to arsenate. Values are given as means (SE) all with a sample size of eight.

Temperature (°C)	Nominal exposure concentration (mg As·L^{-1})	Glycogen concentration (mg glucose·g wet weight^{-1}) Day 28	Day 77
Liver glycogen			
5	0	33.3 (3.7)	34.3 (0.3)[b]
5	1.5	29.3 (1.7)[a]	40.7 (0.9)[a]
5	18.0	28.2 (0.3)	31.9 (1.5)
5	36.0	26.1 (3.1)	16.8 (2.9)[b]
15	0	32.8 (0.5)	32.4 (7.7)[c]
15	1.5	36.8 (4.7)	29.5 (3.3)
15	9.0	32.8 (4.4)	27.9 (1.2)
15	18.0	32.9 (2.1)	8.2 (1.0)[c]
Muscle glycogen			
5	0	5.1 (0.1)	3.1 (0.3)
5	1.5	4.0 (0.8)	2.2 (0.3)
5	18.0	2.9 (0.5)	2.1 (0.5)
5	36.0	0.8 (0.2)[d]	1.1 (0.1)[d]
15	0	7.6 (2.4)	1.6 (0.2)
15	1.5	7.7 (0.6)	2.4 (0.2)
15	9.0	5.8 (1.3)	2.3 (0.4)
15	18.0	4.7 (0.3)	2.0 (0.5)

[a,b,c]Common superscripts denote significant differences ($P<0.05$).

[d]Significantly different from all other data ($P<0.05$).

muscle glycogen available than they had on day 28. Time had no significant effect on muscle glycogen for the 5°C treatments. The mean muscle glycogen levels shown by the 5°C high-arsenate treatment on day 28 (0.8 mg·g^{-1}) and day 77 (1.0 mg·g^{-1}) were significantly lower than the means shown at either time by all other treatments.

The major pathological lesion evident in the organs sampled was a hardening and thickening of the gall bladder wall, a lesion which occurred only in the fish from the high treatments at both temperatures. Four of the eight trout sampled at 15°C showed this lesion, while it was evident in only two of the eight at 5°C. The only other histological abnormality found was a diffuse micro- and macrovesicular lipidosis of the liver in the high-arsenate fish at 5°C. However, at 15°C, the frequency of micro- and macrovesicular lipidosis was evident in equal proportions for all treatments. All kidney, spleen, muscle, and gill preparations were found to be normal for all treatment and temperatures.

Discussion

When our chronic tests were completed at water concentrations based upon a percentage of the 144-h LC50, rainbow trout were more sensitive to arsenate at 5°C than at 15°C. While this is contrary to our previous work on acute toxicity (McGeachy and Dixon 1989), it is based on the whole-body arsenic concentration and not on the waterborne arsenic concentration.

The most easily measured and consistent indicator of toxicity was mortality. The fish of the high-arsenate treatment (36 mg·L^{-1}) at 5°C suffered the highest mortality rate; deaths started within 7 d of exposure and continued relatively consistently throughout the 11-wk study. A similar delayed and protracted mortality has been reported for arsenite (Rankin 1987), although it did not start to occur until the fifth week of exposure. The lingering mortality indicates that, relative to those at 15°C, the fish at 5°C did not reach a threshold of mortality (incipient lethal level; ILL) during our previously reported acute study. Work by Hodson and Sprague (1975) found that the time to reach the ILL for zinc with Atlantic salmon (*Salmo salar*) changed from 4 d at 15°C to 14 d or more at 5°C. A similar case can also be made for antimony. The LC50 for antimony over 30 d with rainbow trout halved from 37 mg·L^{-1} at 144 h to 16 mg·L^{-1} at 16 d (Doe et al. 1987). So it appears that the time to reach an ILL for arsenate, if one is indeed attained, may be dependent on the temperature regime.

It is apparent that the effect of water temperature on arsenic accumulation is exposure-concentration dependent, and that expressing the waterborne concentrations relative to acute toxicity (high arsenate, 0.30 of the 144-h LC50) will mask the temperature effects. Although the whole-body arsenic concentrations shown by the high-arsenate treatment fish at 5 (36 mg·L^{-1}) and 15°C (18 mg·L^{-1}) were essentially identical, the concentrations in trout exposed to the same absolute concentration (18 mg·L^{-1}) were highly temperature dependent. The trout at 15°C took up more arsenate, which is consistent with the general principle that more toxicants are taken up as the water temperature increases (Cairns et al. 1975). However, the sensitivity (mortality) of the trout with similar body concentrations of arsenic (high-arsenate treatments) was greater at the lower water temperature. An explanation for this apparent contradiction may lie with differences in the forms of arsenic present at the two temperatures. In both fish (Spehar et al. 1980) and mammals (Crecelius 1977), metabolically transformed organoarsenicals are generally less toxic than inorganic arsenic, and are in fact considered to be detoxification products. Not only are the organoarsenicals less toxic, they are also more rapidly excreted (Tam et al. 1978). It is possible that the fish at 15°C, by virtue of a higher metabolic rate, would be in a better position to convert a higher proportion of the arsenate to a less toxic organoarsenical product.

The whole-body arsenic concentrations in moribund trout indicate that a critical internal concentration is reached before toxicant insult and death occur. This threshold concentration (4.0–6.0 μg As·g wet weight^{-1}) is independent of temperature, and to a lesser extent, waterborne arsenic concentration and time. Although well established in mammalian toxicology, the application of the concept that a relationship exists between internal concentration, site of action, and toxic effect is rela-

tively new to aquatic toxicology (McCarty et al. 1989). Recent work with organics and some metals has found that death occurs when a chemical reaches a critical concentration inside the organism (Connolly 1985; Van Hoogen and Opperhuizen 1988). If a relationship between internal concentration, site of action, and toxic effect can be obtained for a chemical, then prediction of the toxicity associated with a given body concentration of that chemical will follow.

Growth and development represent the sum of many interacting processes which can be influenced by toxicants (Brett 1971; Woltering 1984). Relative to most metals, arsenate's effect on growth is subtle. While the effects on mortality and accumulation were statistically significant within the first 2 wk of exposure, arsenate's effects on wet and dry weight did not become significant until well after 28 d. This indicates a narrow range between chronic effects on growth and lethality, an observation substantiated by the lack of a significant arsenate impact on condition factor. A narrow range between an impact on growth and lethality has also been reported for rainbow trout (Rankin 1987) and bluegill sunfish, *Lepomis macrochirus* (Gilderhus 1966), exposed to arsenite.

The liver glycogen results show a trend similar to that of wet and dry weight, in that the toxic effects of arsenate did not reduce liver glycogen levels until well after the onset of mortality. By day 77, however, the levels of high-arsenate fish at both 5 and 15°C were significantly depleted. The temporal correlation of the depressed weights and liver glycogen reserves is indicative of the depressed energy status of the fish. Glycogen has long been known as the storage unit of carbohydrates which can be mobilized via glycolysis as glycogen for energy and growth. During glycolysis, arsenate resembles and replaces phosphate in forming an unstable arsenophosphoglycerate molecule. This compound is rapidly hydrolysed so that glycolysis can proceed, but with the formation of one less ATP (Stryer 1980). Since muscle glycogen is used primarily for burst swimming energy (Hochachka and Somero 1984), it is highly unlikely that reductions seen here would affect growth. It is interesting to note that Speyer (1975) showed a reduction in swimming capacity of rainbow trout after a 3-wk exposure to sublethal levels of arsenite.

The marginally greater hepatosomatic index (HSI) in fish at 5°C, relative to those at 15°C, is consistent with the slightly higher glycogen reserves evident in the 5°C fish (Dixon and Hilton 1985). Although changes in the hepatosomatic index have been used to assess liver stress in fish during toxicant exposure (Johnson 1968; Hickie et al. 1989), the absence of an effect here is not surprising in view of the apparent subtlety of chronic arsenate toxicity. The splenosomatic index (SSI) was measured during this work as a potential indicator of arsenate impact on haematopoiesis (NRCC 1978; Olademeji 1980). The lack of a response, along with the normal histological appearance of the spleen through all treatments, suggests that arsenate has no major effect on haematopoietic function.

With the exception of the gall bladder, histological findings were normal for all treatments. The high incidence of the gall bladder lesion may have been the result of elevated levels of arsenic metabolites accumulating there (Sorenson et al. 1979) due to the elimination of arsenic via the biliary-faecal route (Penrose 1975). Similar gross and microscopic lesions were observed by Rankin (1987) and Cockel and Hilton (1988). How this lesion affects the overall health of the organism and its ability to handle arsenic is unknown.

In summary, rainbow trout tested at 5°C were more sensitive to chronic arsenate exposure than those tested at 15°C. The combination of the early mortality response and the delayed reduction in weight gain at the highest arsenate exposure concentration exemplifies the subtle margin between the lethal and sublethal effects of arsenic. Effects on wet and dry weight were only significant after 78 d of arsenate exposure and any effects on liver and muscle glycogen were minimal. While the pattern and level of arsenic accumulation (whole-body arsenic concentration) between temperatures was similar throughout the experiment, mortality was greatest at 5°C. This type of response could be due to poor detoxification mechanisms within the trout held at the lower water temperature. It is hypothesized that the ratio of organic arsenic to inorganic arsenic is greater at 15°C. This would allow for greater arsenic tolerance when total arsenic body burdens would suggest otherwise. A closer examination of the constituents of the total arsenic content is needed to verify this hypothesis.

Acknowledgements

This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic (G1159) and Operating (8511) grants to D. G. Dixon, and by an NSERC Postgraduate Scholarship to S. M. McGeachy. Technical assistance was provided by E. J. Coker, P. A. Miller, K. R. Munkittrick, and M. G. Rankin.

References

Brett, J. R. 1971. Temperature, p. 515–615. *In* O. Kinne [ed.] Marine Ecology. Vol. 1, Environmental Factors. Wiley-Interscience, New York, N.Y.

Cairns, J., Jr., A. G. Heath, and B. C. Parker. 1975. Temperature influence on chemical toxicity to aquatic organisms. J. Water Pollut. Control. Fed. 47: 267–280.

Cockel, K. A., and J. W. Hilton. 1988. Preliminary investigations on the comparative chronic toxicity of four dietary arsenicals to juvenile rainbow trout (*Salmo gairdneri* R.). Aquat. Toxicol. 12: 73–82.

Connolly, J. P. 1985. Predicting single-species toxicity in natural water systems. Environ. Toxicol. Chem. 4: 573–582.

Crecelius, E. A. 1977. Changes in the chemical speciation of arsenic following ingestion by man. Environ. Health Perspect. 19: 147–150.

Dixon, D. G. and J. W. Hilton. 1985. Effects of available dietary carbohydrate and water temperature on the chronic toxicity of waterborne copper to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 42: 1007–1013.

Doe, K. G., W. R. Parker, S. J. Ponsford, and J. D. A. Vaughan. 1987. The acute and chronic toxicity of antimony to *Daphnia magna* and rainbow trout, p. 40–43. *In* J. S. S. Lakshminarayana [ed.] Proceedings of the Thirteenth Annual Aquatic Toxicity Workshop: November 12–14, 1986, Moncton, New Brunswick, Can. Tech. Rep. Fish. Aquat. Sci. 1575.

Ferguson, J. F., and J. Gavis. 1972. A review of the arsenic cycle in natural waters. Water Res. 6: 1259–1274.

Fry, F. E. J. 1971. Effects of environmental factors on the physiology of fish, p. 1–98. *In* W. S. Hoar and D. J. Randall [ed.] Fish Physiology, Vol. VI, Environmental Relations and Behaviour. Academic Press, London, U.K.

Gilderhus, P. A. 1966. Some effects of sublethal concentrations of sodium asenite on bluegills and the aquatic environment. Trans. Am. Fish. Soc. 95: 289–296.

Gulledge, J. H., and J. T. O'Connors. 1973. Removal of arsenic (V) from water by adsorption on aluminum and ferric hydroxides. J. Am. Water Works Assoc. 65: 548–552.

Hickie, B. E., D. G. Dixon, and J. F. Leatherland. 1989. The influence of the dietary carbohydrate: lipid ratio on the chronic toxicity of sodium pentachlorophenate to rainbow trout (*Salmo gairdneri* Richardson). Fish Physiol. Biochem. 6: 175–185.

Hilton, J. W., and S. J. Slinger. 1981. Nutrition and feeding of rainbow trout. Can. Spec. Publ. Fish. Aquat. Sci. 55: 1–15.

Hochachka, P. A., and G. N. Somero. 1984. Biological adaptation. Princeton University Press, Princeton, N.J. 537 p.

HODSON, P. V., AND J. B. SPRAGUE. 1975. Effect of temperature on the acute toxicity of zinc to Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Canada 32: 1–10.

JOHNSON, D. W. 1968. Pesticides and fishes — A review of selected literature. Trans. Am. Fish. Soc. 97: 398–424.

LEMKE, A. E., W. A. BRUNGS, AND B. J. HALLIGAN. 1978. Manual for construction and operation of toxicity testing proportional diluters. Publ. No. EPA-600/3-78-072. U.S. Environmental Protection Agency, Washington, D.C. 81 p.

MCCARTY, L. S., G. W. OZBURN, A. D. SMITH, A. BHARATH, D. ORR, AND D. G. DIXON. 1989. Hypothesis formulation and testing in aquatic bioassays: a deterministic model approach. Hyrobiologia 189/190: 533–542.

MCGEACHY, S. M., AND D. G. DIXON. 1989. The impact of temperature on the acute toxicity of arsenate and arsenite to rainbow trout (*Salmo gairdneri*). Ecotoxicol. Environ. Saf. 17: 86–93.

MORITO, C. L. H., D. H. CONRAD, AND J. W. HILTON. 1986. The thiamin deficiency signs and requirements of rainbow trout (*Salmo gairdneri* Richardson). Fish Physiol. Biochem. 1: 93–104.

NATIONAL RESEARCH COUNCIL OF CANADA (NRCC). 1978. Effects of arsenic in the Canadian environment. Publ. No. 15391, National Research Council of Canada, Ottawa, Ont. 363 p.

OLADEMEJI, A. A. 1980. Bioaccumulation and metabolism of arsenic by rainbow trout. Ph.D. thesis, University of Ottawa, Ottawa, Ont. 137 p.

PASSONEAU, J. V., AND V. R. LAUDERDALE. 1974. A comparison of three methods of glycogen measurement in tissues. Anal. Chem. 60: 405–412.

PENROSE, W. R. 1975. Biosynthesis of organic arsenic compounds in brown trout (*Salmo trutta*). J. Fish. Res. Board Can. 32: 2385–2390.

RANKIN, M. G. 1987. Acute and chronic toxicity of waterborne inorganic trivalent arsenic to rainbow tout (*Salmo gairdneri*). M.Sc. thesis. University of Waterloo, Waterloo, Ont. 92 p.

SORENSON, E. M. B., R. E. HENRY, AND R. RAMIREZ-MITCHELL. 1979. Arsenic accumulation, tissue distribution and cytotoxicity in teleosts following indirect aqueous exposures. Bull. Environ. Contam. Toxicol. 21: 162–169.

SPEHAR, R. L., J. T. FIANDT, R. L. ANDERSON, AND D. L. DEFOE. 1980. Comparative toxicity of arsenic compounds and their accumulation in invertebrates and fish. Arch. Environ. Contam. Toxicol. 9: 53–63.

SPEYER, M. R. 1975. Some effects of chronic combined arsenic and cyanide poisoning on the physiology of rainbow trout. M.Sc. thesis. Concordia University, Montreal, Qué. 76 p.

SPRAGUE, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

1985. Factors that modify toxicity, p. 124–163. *In* G. M. Rand and S. R. Petrocelli [ed.] Fundamentals of aquatic toxicology: methods and applications. Hemisphere Publishing Corp., New York, N.Y.

STEELE, R. G. D., AND J. H. TORRIE. 1980. Principles and procedures of statistics: a biometrical approach, 2nd ed. McGraw–Hill, New York, N.Y. 481 p.

STRYER, L. 1980. Biochemistry, 2nd ed. W. H. Freeman and Co., San Francisco, CA. 949 p.

TAM, K. H., S. M. CHARBONNEAU, F. BRYCE, AND G. LACROIX. 1978. Separation of arsenic metabolites in dog plasma and urine following intravenous injection of ^{74}As. Anal. Biochem. 86: 505–511.

VAN HOOGEN, G., AND A. OPPERHUIZEN. 1988. Toxicokinetics of chlorobenzenes in fish. Environ. Toxicol. Chem. 7: 213–219.

WOLTERING, D. M. 1984. The growth response in fish chronic and early life stage toxicity tests: a critical review. Aquat. Toxicol. 5: 1–21.

Comparison of Methods for Estimating Mixed Stock Fishery Composition

R. B. Millar

Science Branch, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, Newfoundland A1C 5X1 Canada

Millar, R. B. 1990. Comparison of methods for estimating mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 47: 2235–2241.

Given information on fish of known origin, and a random sample from the mixed stock fishery, the composition of that mixed fishery may be estimated in a number of ways. This study compares the performance of four classification-based estimators and a maximum likelihood estimator. Theoretical considerations show that the maximum likelihood estimator makes better use of the information contained in the mixed fishery sample. However, the classification estimators are shown to be more robust to violations in some of the model assumptions. Scale data from four regional stock groups of chinook salmon (*Oncorhynchus tshawytscha*) were used in an applied comparison of the five estimators. The results suggest that the maximum likelihood estimator performs best in practice.

Compte tenu de l'information sur le poisson d'origine connue et d'un échantillonnage au hasard dans les pêches de stocks divers, la composition de ces pêches diverses peut être évaluée par un certain nombre de méthodes. Cette étude se penche sur l'efficacité comparée de quatre estimateurs fondés sur la classification et d'un estimateur du maximum de vraisemblance. Des considérations théoriques montrent que l'estimateur du maximum de vraisemblance fait une meilleure utilisation de l'information contenue dans l'échantillon de pêches diverses. Cependant, les estimateurs fondés sur la classification apparaissent comme plus résistants aux effets d'irrégularités concernant certaines des hypothèses de modèle. Les données d'échelle provenant de quatre (4) groupes régionaux de stocks de saumon quinnat (*Oncorhynchus tschawytscha*) ont servi aux fins d'une comparaison appliquée de cinq (5) estimateurs. Les résultats portent à croire que, dans la pratique, c'est l'estimateur du maximum de vraisemblance qui est le plus efficace.

Received September 14, 1989
Accepted May 10, 1990
(JA305)

Reçu le 14 septembre 1989
Accepté le 10 mai 1990

Five different estimators of mixed fishery composition were compared. Four of these estimators employ a classification step (Worlund and Fredin 1962; Cook and Lord 1978; Pella and Robertson 1979; Cook 1982, 1983) and the fifth estimator is based on maximum likelihood (Milner et al. 1981, 1983; Fournier et al. 1984; Millar 1987). A linear regression estimator has been used in composition problems using discrete data (Okazaki 1985) but it is easily shown to be an inappropriate method (Mulligan et al. 1988) and is not included in this study.

At present, researchers typically use a classification estimator when the data are continuous (e.g. Myers et al. 1984) while the maximum likelihood estimator is used with discrete data, such as genetic data (Milner et al. 1981, 1983). This division of methods on the basis of data type is solely for reasons of computational convenience and regardless of data type the researcher has the option to use either type of estimator.

Millar (1987) and Wood et al. (1987) have made elementary comparisons between the classification and maximum likelihood estimators and have shown that the maximum likelihood estimator makes better use of the information contained in the sample from the mixed fishery population. However, as explained later, the classification estimators are likely to be more robust to violations of assumptions and therefore it is not clear which type of estimator is to be preferred in practice.

This paper makes a comprehensive comparison of the estimators on both theoretical and practical grounds. Theoretical considerations show that, of the four classification estimators studied, only two make sense statistically and therefore the practical comparison is between these two classification estimators and the maximum likelihood estimator. The practical comparison is achieved through the simulation of a four stock mixed fishery. The simulated mixed fishery samples are free from distributional assumptions since they are generated by sampling from real chinook salmon (*Oncorhynchus tshawytscha*) data.

In this study the mixed fishery population is a mixture of fish from different stocks (origins), though more generally these methods can be used when the mixture is defined by any other factor that partitions the fishery. (For example, to estimate the age composition of a fishery.) It is required that information is known about the distribution of the measured variables within each of the stocks. This information (the baseline data) is typically obtained by collecting data from fish of known origin.

For each fish in the mixed fishery sample, all five estimators require calculation of the likelihood of observing that fish (i.e. the measurements made on that fish) given that it is from stock j. Denote these by

x_{ij} = likelihood of observing the measurements made on fish i
given that it is from stock j, $i = 1,\ldots,N$, $j = 1,\ldots,S$,

where N is the size of the mixed fishery sample and S is the number of stocks comprising the mixed fishery. In practice, the x_{ij}'s are estimated by using the baseline data to estimate the distribution of the measured variables within each stock.

The classification-based methods use $x_{ij}, j=1,\ldots,S$ to assign fish i to one of the S stocks, typically by assigning it to the stock for which x_{ij} is highest. The maximum likelihood method uses the x_{ij}'s directly as arguments in constructing the likelihood function for the entire mixed fishery sample.

The four classification estimators are described in the next section. One of the classification estimators employs a maximum likelihood step after classification of the mixed fishery sample. To avoid confusion, this will be called the maximum classification-likelihood estimator and henceforth the (non-classifying) maximum likelihood estimator will be referred to as the direct maximum likelihood estimator. The classification rule subsection outlines some of the options available to the practitioner and also explains the manner in which the classification estimators are robust.

Classification-Based Estimators

In describing the classification-based estimators, it is assumed only that some classification rule (see below) has been specified, i.e. that one has a procedure for classifying a fish on the basis of the measured variables. The corresponding classification matrix will be denoted by Φ, where the element ϕ_{ij} of Φ is the probability that a randomly chosen fish from stock j is classified to stock i.

The vector $\Theta = (\theta_1, \theta_2,\ldots,\theta_S)^T$ will denote the composition of the mixed fishery. Let r be the vector of proportions that would be obtained if the entire mixed fishery population were classified, i.e. r_j is the proportion of the mixed fishery that would be classified as stock j, $j=1,\ldots,S$. The proportions of the mixed fishery sample classified to each stock will be denoted by $\hat{r}$.

The Estimators

1. Raw classification proportions estimator, $\hat{\Theta}_1 = \hat{r}$.

As explained later, $\hat{\Theta}_1$ is not a sensible estimator of Θ, but it is a good estimator of r and this property is utilized by $\hat{\Theta}_2$ below.

2. Cook and Lord's (1978) corrected classification estimator, $\hat{\Theta}_2$ (see also Pella and Robertson 1979).

Cook and Lord (1978) realized that r is a function of Θ, given by

$$r = \Phi\Theta$$

and therefore (assuming that Φ is invertible),

$$(1) \quad \Theta = \Phi^{-1}r.$$

Replacing the quantities on the right side of expression (1) by their estimated values gives,

$$\hat{\Theta}_2 = \hat{\Phi}^{-1}\hat{r},$$

provided the estimated classification matrix $\hat{\Phi}$ is invertible.

For an estimated vector of composition to make sense, its elements must be non-negative and sum to 1. $\hat{\Theta}_2$ has element sum 1 (Millar 1987), but it can violate the non-negativity condition. This deficiency leads to the estimator below.

3. Cook's (1983) constrained corrected classification estimator, $\hat{\Theta}_3$.

If $\hat{\Theta}_2$ is non-negative, then $\hat{\Theta}_3 = \hat{\Theta}_2$. Otherwise the non-negative $\hat{\Theta}_3$ is obtained from $\hat{\Theta}_2$ by a simple iterative scheme (Cook 1983) within a finite number of iterations.

4. Maximum classification-likelihood estimator, $\hat{\Theta}_4$ (Millar 1987).

The likelihood of observing the raw classification proportions $\hat{r}$ can be expressed as a function of Φ and Θ. By replacing Φ with $\hat{\Phi}$ in the likelihood function, it is then possible to maximize over Θ to obtain $\hat{\Theta}_4$. The maximization is implicitly constrained to non-negative Θ's. Millar (1987) showed that $\hat{\Theta}_4 = \hat{\Theta}_2$ when the latter is non-negative.

When $\hat{\Theta}_2$ has negative components, then instead of computing $\hat{\Theta}_3$ or $\hat{\Theta}_4$ one could calculate a new $\hat{\Theta}_2$ by repeating the analysis after first removing from the mixed fishery model those stocks estimated to have negative contribution. This estimator was not considered here due to its impracticality and difficulty of implementation. Moreover, there is no guarantee that the new $\hat{\Theta}_2$ will be non-negative and it may be necessary to repeat the procedure again.

The Classification Rule

In practice the classification rule is derived from the baseline data and must be chosen so as to ensure good performance from the estimators. Intuitively, the better the classification rule, the better the performance of the estimators. This can be shown quantitatively by examining the variance of the Cook and Lord estimator $\hat{\Theta}_2$ (ignoring possible problems with non-negativity). If $\Sigma_{\hat{r}}$ denotes the covariance matrix of $\hat{r}$ then the covariance matrix of $\hat{\Theta}_2$ is approximated by

$$(2) \quad \Sigma_{\hat{\Theta}_2} = \hat{\Phi}^{-1}(\Sigma_{\hat{r}} + \Sigma_b)(\hat{\Phi}^{-1})^T$$

where Σ_b is a term arising from the baseline sampling (Pella and Robertson 1979).

When the measured variables contain little information to distinguish between stocks, then the elements $\hat{\phi}_{ij}$ of $\hat{\Phi}$ will all be close to $1/S$. Thus $\hat{\Phi}$ will be near singular and $\hat{\Phi}^{-1}$ will contain some elements of high magnitude. From expression (2) it follows that $\Sigma_{\hat{\Theta}_2}$ will also have components of high magnitude and hence that $\hat{\Theta}_2$ will have high variance. When the measured variables contain good information to distinguish between stocks then Φ will be close to the $S \times S$ identity matrix. Then Φ^{-1} will also be close to the identity and $\Sigma_{\hat{\Theta}_2}$ will have elements of relatively low magnitude.

It is not apparent from (2) how the classification rule should be constructed to minimize the "magnitude" of $\Sigma_{\hat{\Theta}_2}$. Moreover, $\Sigma_{\hat{r}}$ and Σ_b are functions of the unknown mixed fishery composition. The usual classification rule of assigning fish i to the stock with highest likelihood of that fish is a logical choice. It is commonly known as the optimal classification rule because it minimizes the probability of misclassification. Cook (1982) suggests using artificial prior distributions to modify the classification rule so that the classification matrix is made more symmetric, though no justification for this modification is given.

With multivariate normal data the optimal classification rule can be implemented by most statistical software packages (BMDP, SAS, SPSS, etc.) If it is assumed that the variables have a covariance matrix that is common across all of the stocks then (to within a constant) the calculation of $\log_e(x_{ij})$, $j=1,\ldots,S$, is reduced to the evaluation of linear functions of the measured variables. The procedure of assigning fish i to the stock for which x_{ij} (or equivalently $\log_e(x_{ij})$) is highest is thus reduced to the comparison of S linear functions of fish i's measurements, and consequently is called linear discriminant analysis (LDA) (Lachenbruch 1975; Johnson and Wichern 1982).

Dispensing with the assumption of common covariance matrices leads to quadratic discriminant analysis (QDA) since $\log_e(x_{ij})$ is then a quadratic function of the measured variables.

For multivariate normal data Φ is usually estimated by the statistical software performing the LDA or QDA. Some software packages calculate Φ by classifying the learning sample (the baseline data) and setting $\hat{\phi}_{ij}$ equal to the proportion of the stock j baseline classified to stock i. This tends to under-estimate the misclassification probabilities, though not seriously except when the size of the baseline data set is very small. The more sophisticated software calculates a better estimate of Φ by using a leave-one-out approach.

Using the classification estimators when the measured variables are not multivariate normal typically requires customized software to calculate x_{ij} and Φ. For example, with genetic variables the baseline data may consist of estimated allele frequencies for each of the stocks. The likelihoods x_{ij} can be calculated from these frequencies under the assumptions of Hardy–Weinberg equilibrium and independence of loci. Without the convenience of packaged classification software, researchers using discrete data have explored another method, the (direct) maximum likelihood estimator, $\hat{\Theta}_5$.

The consequence of using the wrong distributional assumptions when calculating the likelihoods x_{ij} is that the classification rule will not perform as well as it could. However, provided the mixed fishery sample is representative of the mixed fishery population, the estimated classification matrix Φ will still be a good estimator of Φ and the classification estimators will remain valid. The covariance matrix $\Sigma_{\hat{\Theta}_2}$ will reflect the poor performance of the classification rule by having elements of high magnitude. In contrast, the direct maximum likelihood estimator could be completely corrupted by bad distributional assumptions.

Direct Maximum Likelihood Estimator

This estimator, $\hat{\Theta}_5$, is defined as the composition that maximizes the likelihood of the entire mixed fishery sample. Millar (1987) explains that this likelihood can be written as

$$(3)\quad L = \prod_{i=1}^{N} \sum_{j=1}^{S} x_{ij}\theta_j.$$

As with the classification estimators, the practitioner must decide what assumptions to make when calculating the x_{ij}'s from the baseline data. For example, if the data are continuous, are they approximately multivariate normal and is it reasonable to assume a common covariance matrix across all stocks?

The log-likelihood function $\log_e L$ is almost guaranteed to be a strictly concave function of Θ (Millar 1987), in which case $\hat{\Theta}_5$ is unique. Calculation of $\hat{\Theta}_5$ is a constrained nonlinear maximization problem. Fournier et al. (1984) use a quasi-Newton maximization algorithm and Pella and Milner (1987) propose an iteratively reweighted least squares approach. Milner et al. (1983) and Millar (1987) present the EM (expectation-maximization) algorithm (Dempster et al. 1977; Wu 1983) as a method to maximize $\log_e L$ that can be programmed in a few lines of FORTRAN. Using the EM algorithm, the program code required to calculate $\hat{\Theta}_5$ is considerably simpler than that required to calculate a classification based estimator.

Theoretical Comparison of Estimators

The relative performance of the estimators can be deduced in part on theoretical grounds. Some theoretical considerations are summarized below and more detail can be found in Millar (1987). Since Cook and Lord's $\hat{\Theta}_2$ can be non-negative, it is not a well defined estimator of composition and therefore is not considered further.

1. The proportions of mixed sample fish classified to each stock, $\hat{r}$, give the raw classification proportions estimator $\hat{\Theta}_1$. This estimator is flawed because it does not take into account the probability of a mixed sample fish being misclassified. For any given classification rule $\hat{\Theta}_1$ is an unbiased estimator of r and therefore the bias of $\hat{\Theta}_1$ (as an estimator of the composition Θ) is $r-\Theta$.
2. The estimators $\hat{\Theta}_3$, $\hat{\Theta}_4$ and $\hat{\Theta}_5$ are all slightly biased and the bias tends to be greatest when some of the S stocks in the mixed fishery are low or non-contributors. The bias typically decreases quickly as the size of both the baseline and mixed samples increases. In contrast, the bias of $\hat{\Theta}_1$ can be extreme and is not reduced by increased sample sizes.
3. The direct maximum likelihood estimator $\hat{\Theta}_5$ makes better use of the information in the mixed fishery sample than the classification based estimators. This is because classification of a fish (using the optimal classification rule) only indicates which stock had the highest likelihood of that fish. To illustrate, consider a fish with measurements that are quite typical (i.e. highly likely) for fish from stock 1, but are highly unusual for fish from any other stock. This fish would be classified to stock 1 and its classification would most likely be correct. In the other extreme, consider a fish with measurements that are close to equally likely across all of the stocks, but with likelihood that is slightly higher for stock 1. This fish is also classified to stock 1, but in this case the classification is unreliable and has high probability of being wrong. The former fish is therefore more informative than the latter. This difference in reliability of each fish's classification is not used by the classification-based methods since the classification matrix gives only average (over all fish in the stock) misclassification probabilities and provides no indication as to how well any particular fish has been classified. In contrast, the direct maximum likelihood method uses all of the information contained in the likelihood values.
4. The classification-based methods may be more robust than the direct maximum likelihood method to anomalies in the baseline data since they rely only on Φ being a good estimate of Φ. Possible anomalies include the presence of outliers or gross violations in the assumptions (e.g. that the measured variables are multivariate normal or that loci are independent and in Hardy–Weinberg equilibrium).
5. Estimators $\hat{\Theta}_3$ and $\hat{\Theta}_4$ will be different when $\hat{\Theta}_2$ has negative elements. Cook's (1983) estimator $\hat{\Theta}_3$ is simply a geometric modification of $\hat{\Theta}_2$ and it makes no reference to the actual composition estimation problem at hand. The maximum classification-likelihood estimator $\hat{\Theta}_4$ fully utilizes the estimated classification matrix and hence should be better.

Properties 3 and 4 above show that the classification-based and direct maximum likelihood estimators have their respective advantages and disadvantages and therefore comparisons with real data are required to assess their relative performance in practice. Properties 1 and 2 show that $\hat{\Theta}_1$ can be eliminated from the comparison, leaving just $\hat{\Theta}_3$, $\hat{\Theta}_4$ and $\hat{\Theta}_5$.

Consideration must also be given to the problem of estimating the covariance matrix of the composition estimator. There are no analytic methods that fully answer this problem. The

TABLE 1. Mean and standard deviation (in parentheses) of eight scale variables measured on chinook salmon from the 1971 brood year. Sample sizes from the regions ASIA, WEST, CENT, and SEBC were 161, 200, 122, and 195, respectively.

Variable	ASIA	WEST	CENT	SEBC
1	147.5 (13.0)	160.7 (13.1)	150.6 (13.4)	137.8 (11.9)
2	1049 (145)	1158 (180)	1185 (171)	1225 (188)
3	28.68 (2.83)	30.56 (3.09)	29.69 (3.15)	32.60 (3.62)
4	107.8 (13.7)	118.4 (15.2)	121.6 (19.2)	107.3 (13.6)
5	.0621 (.0093)	.0549 (.0097)	.0596 (.0126)	.0571 (.0103)
6	1656 (216)	1680 (230)	1645 (188)	1502 (180)
7	.8088 (.0255)	.8172 (.0277)	.8080 (.0312)	.7878 (.0352)
8	5750 (724)	6493 (692)	5885 (684)	6173 (679)

asymptotic covariance matrix obtained from maximum likelihood theory (calculated by inverting the information matrix) can be used to obtain estimated covariance matrices for $\hat{\Theta}_4$ or $\hat{\Theta}_5$, but it only takes into consideration the sampling randomness in the mixed fishery sample and ignores the randomness in the baseline data. Also, this approach can not handle the non-negativity constraint, and is prone to over-estimate the covariances (Millar 1987). The over-estimation can be serious, depending on the number of stocks that are estimated to have low or no contribution to the mixed fishery. Millar's (1987) infinitesimal jackknife (for $\hat{\Theta}_4$ and $\hat{\Theta}_5$) does handle the non-negativity constraint but it also takes into account only the sampling randomness in the mixed fishery sample. When $\hat{\Theta}_2$ is non-negative, one could use the estimated covariance matrix $\Sigma_{\hat{\Theta}_2}$ in (2) as an estimator of the covariance matrix for $\hat{\Theta}_3$ and $\hat{\Theta}_4$ because then $\hat{\Theta}_2 = \hat{\Theta}_3 = \hat{\Theta}_4$. However, a covariance estimator that is dependent on $\hat{\Theta}_2$ being non-negative is hardly practical. Also, as with the asymptotic covariance matrix estimator, the consequence of ignoring the non-negativity constraint is that it tends to over-estimate. No matter which composition estimator is used, the best estimator of its covariance matrix is probably obtained by bootstrapping.

Simulation Study and Results

The mixed fishery used here is comprised of four regional stock groups of age 1.2 chinook salmon (*Oncorhynchus tshawytscha*) as defined by Myers et al. (1984). The regions are Asia, Western Alaska, Central Alaska, and Southeastern Alaska/British Columbia (ASIA, WEST, CENT, and SEBC respectively). Scale measurements from fish in the 1971 and 1977 brood years were used. The 1971 baseline data consist of eight variables (Table 1) measured on 161, 200, 122, and 195 fish from the ASIA, WEST, CENT, and SEBC stock groups respectively. The 1977 baseline data include twelve variables measured on 200, 199, 199, and 198 fish respectively.

The simulation programs use the given baseline data to define a hypothetical mixed fishery population. When constructing a mixed fishery sample the baseline is sampled with replacement. The number of fish sampled from the four stock group baselines is a random vector of length four. This vector has a multinomial distribution, with parameters defined by the desired size of the mixed fishery sample and the specified mixed fishery composition. To emulate the randomness involved in the collection of baseline data, the programs resample from the given baseline (with replacement) to obtain a new baseline data set.

Linear discriminant analysis was used by Myers et al. (1984) on the basis of performance, convenience, and compatability with the work of other researchers. They used the BMDP statistical package to perform the classification calculations. The simulation programs perform the same classification calculations, but also save the likelihood values, x_{ij}, $i=1,\ldots,N$, $j=1,\ldots,S$, for use by the direct maximum likelihood estimator. This estimator was calculated by maximizing the likelihood (3) using the EM algorithm.

Simulation results for the Cook and Lord estimator $\hat{\Theta}_2$ are not given since they are without practical interpretation — they would represent the performance of $\hat{\Theta}_2$ under the assumption that one could report, and somehow explain, a negative proportion of a stock. Results for the raw proportions estimator $\hat{\Theta}_1$ are given since they illustrate its unsuitability as a composition estimator (as discussed in the previous section).

Table 2 shows the outcome of a 1971 brood year simulation where the mixed fishery composition of ASIA, WEST, CENT, and SEBC was specified as 0.25, 0.65, 0.10, and 0.00, respectively. (These results were obtained from a multipurpose simulation/bootstrap/analysis program that computes all five estimators (Millar 1990). As used here, in simulation mode, the program requires only baseline data. If both baseline data and mixed fishery sample data are provided, the user can request the multipurpose program to perform an analysis. In bootstrap mode the program will repeat the analyses with resampled mixed fishery data (and resampled baseline data, if desired), to obtain a non-parametric estimate of the variability in the estimated compositions.) One hundred simulated mixed fishery samples, of 100 fish each, were analyzed. Boxplots of $\hat{\Theta}_3$, $\hat{\Theta}_4$, and $\hat{\Theta}_5$ (Fig. 1) show the distributions of the estimated ASIA and WEST contributions to be close to symmetric. The estimates for the two stock groups of lowest contribution, CENT and SEBC, have a right-skewed distribution. This is a consequence of the non-negativity constraint.

The baseline data were not resampled in the above study, and therefore the classification rule remained fixed throughout the one hundred simulations. The classification matrix was estimated by classifying the baseline data, i.e. $\hat{\phi}_{ij}$ is the proportion of fish in the stock j baseline that were classified to stock i. It is interesting to note that, in this case, classification of the baseline data is equivalent to classification of the (hypothetical) population. This is because, for the purposes of the simulations, the baseline data define the population. That is, $\hat{\Phi} = \Phi$. Here,

$$\Phi = \begin{pmatrix} .7081 & .1300 & .1311 & .0513 \\ .1615 & .7950 & .1230 & .0103 \\ .1118 & .0750 & .6148 & .1897 \\ .0186 & .0000 & .1311 & .7487 \end{pmatrix}.$$

Recall that the expected value of the raw classification proportions estimator $\hat{\Theta}_1$ is r (the proportions of the mixed fishery population that would be classified to each stock group). This is demonstrated by the mean of the one hundred estimated $\hat{\Theta}_1$'s (Table 2), which is very close to its expected value $r = \Phi\Theta = (0.2746, 0.5694, 0.1382, 0.0178)^T$.

Comparison of estimators $\hat{\Theta}_3$, $\hat{\Theta}_4$, and $\hat{\Theta}_5$ (Table 2) shows that the direct maximum likelihood estimator $\hat{\Theta}_5$ has lowest mean square error for all four components (ASIA, WEST, CENT, and SEBC) of the composition vector. Over a dozen further simulation runs (of 100 simulations each), with different true compositions, it was always better in at least two components. Moreover, in every simulation run, the total mean squared error when summed over the four component stocks was smallest for the direct maximum likelihood estimator.

TABLE 2. The means, standard deviations, and mean squared errors of the composition estimators over 100 simulations using the 1971 brood year chinook salmon data. In each simulation a 100 fish random sample was generated from a mixed fishery population with composition (0.25, 0.65, 0.10, 0.00). The baseline data were not resampled.

	Regional stock group	Raw classification proportions	Cook's (1983) constrained corrected	Maximum classification-likelihood	Direct maximum likelihood
Means	ASIA	.2804	.2562	.2604	.2607
	WEST	.5680	.6435	.6523	.6532
	CENT	.1361	.0945	.0812	.0853
	SEBC	.0155	.0058	.0060	.0008
Standard deviations	ASIA	.0419	.0729	.0724	.0616
	WEST	.0525	.0827	.0814	.0714
	CENT	.0370	.0674	.0596	.0495
	SEBC	.0114	.0116	.0118	.0036
Mean squared errors	ASIA	.0027	.0054	.0053	.0039
	WEST	.0095	.0069	.0066	.0051
	CENT	.0027	.0046	.0039	.0027
	SEBC	.0004	.0002	.0002	.0000

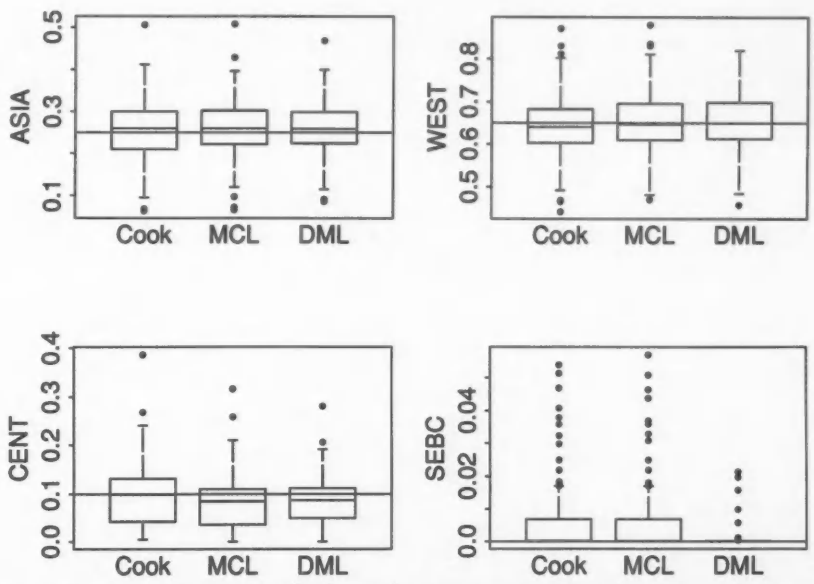

FIG. 1. Boxplots of the estimators $\hat{\Theta}_3$, $\hat{\Theta}_4$ and $\hat{\Theta}_5$ (labelled Cook, MCL, and DML respectively) for 100 simulations using the 1971 brood year chinook salmon data. In each simulation a 100 fish random sample was generated from a mixed fishery population with composition (0.25, 0.65, 0.10, 0.00). The baseline data were not resampled. The middle line in each box is the median value and the top and bottom of the box are the upper quartile and lower quartile, respectively. The height of the box is the interquartile range. The "whiskers" that extend from the top and bottom of each box reach to the furthest value that is within 1.5 times the interquartile range. Any values beyond that are displayed as dots. The median value of the estimated SEBC contribution is 0.00 for all three estimators.

In Table 2 one may note that the raw classification proportions $\hat{\Theta}_1$ did best for estimating the contribution of the Asian group — this is entirely a fortuitous result. The further simulation runs showed that for most mixture compositions the raw proportions were the worst estimator of the Asian contribution.

After performing the above simulations runs, it became apparent that the relative performance of the estimators was highly dependent on the mixed fishery composition. Rather than performing many simulation runs over a range of compositions, a second program was written to randomly choose the mixed fishery composition prior to the generation of each mixed fishery sample. That is, for a run of 100 simulations, the program uses 100 different mixed fishery compositions. It is no longer possible to separate variance from bias since they depend on the particular composition and the program only does one simulation for each composition. The measure of estimator performance used is the average (over the simulations) of the squared differences.

TABLE 3. The means of the squared differences between estimated and true compositions over 100 simulations using the 1971 brood year chinook salmon data. The true composition was randomly generated prior to each simulation. The baseline data were resampled.

Regional stock group	Raw classification proportions	Cook's (1983) constrained corrected	Maximum classification-likelihood	Direct maximum likelihood
100 fish mixed sample				
ASIA	.0093	.0046	.0048	.0036
WEST	.0077	.0038	.0037	.0029
CENT	.0127	.0065	.0064	.0043
SEBC	.0055	.0035	.0034	.0025
400 fish mixed sample				
ASIA	.0057	.0022	.0022	.0016
WEST	.0046	.0016	.0016	.0013
CENT	.0123	.0034	.0034	.0021
SEBC	.0038	.0016	.0017	.0014

TABLE 4. The means of the squared differences between estimated and true compositions over 100 simulations using the 1977 brood year chinook salmon data. The true composition was randomly generated prior to each simulation. The baseline data were resampled.

Regional stock group	Raw classification proportions	Cook's (1983) constrained corrected	Maximum classification-likelihood	Direct maximum likelihood
100 fish mixed sample				
ASIA	.0029	.0029	.0029	.0027
WEST	.0053	.0035	.0034	.0026
CENT	.0061	.0036	.0035	.0022
SEBC	.0034	.0023	.0023	.0018
400 fish mixed sample				
ASIA	.0020	.0009	.0009	.0011
WEST	.0035	.0009	.0009	.0010
CENT	.0048	.0015	.0015	.0007
SEBC	.0025	.0008	.0008	.0006

Four runs of 100 simulations each were performed with the random composition program, two for each of the brood years (Table 3, 4). Within each brood year the two simulation runs used mixed sample sizes of 100 and 400 respectively. In these runs the baseline data were resampled to include the effect on the composition estimators of randomness in the estimation of the likelihoods x_{ij}. The resampled baselines were specified to be the same size as the given baseline, thus mimicking the amount of sampling randomness actually present in the (given) baseline data. Since each simulation generated a new baseline, the program had to perform the linear discriminant analysis and calculate the estimated classification matrix each time. Execution time was increased about threefold compared to the simulations that did not resample the given baseline data.

Without exception, the 1977 brood year simulations (Table 4) had lower average squared differences than the 1971 brood year simulations (Table 3). This is because the 1977 brood year baseline contains more information (12 variables) than the 1971 brood year baseline (8 variables). Also, the 1977 baseline is larger and therefore the randomness in the estimators due to baseline data resampling would be less.

The direct maximum likelihood estimator $\hat{\Theta}_5$ performed best in all four runs and the reduction in average squared error was typically 20–30%. Only in the 1977 brood year run with mixed sample size 400 did direct maximum likelihood not perform best at estimating all four components of the composition vector (Table 4). There the classification estimators $\hat{\Theta}_3$ and $\hat{\Theta}_4$ were better at estimating the contribution from ASIA and WEST with respective decreases in average squared error of 18 and 10%. However, the CENT and SEBC contributions were estimated better by direct maximum likelihood with improvements of 53 and 25%, respectively. As deduced from theoretical considerations, $\hat{\Theta}_1$ is not an appropriate estimator of composition and hence its poor performance.

The two classification estimators $\hat{\Theta}_3$ and $\hat{\Theta}_4$ had almost identical performance and additional comparison would be required to recommend one over the other. In this study, the differences between these two estimators were obscured by the property that much of the time they are equal, since $\hat{\Theta}_2 = \hat{\Theta}_3 = \hat{\Theta}_4$ whenever $\hat{\Theta}_2$ is non-negative. In composition analyses with a greater number of stock groups there would be more chance of $\hat{\Theta}_2$ having negative elements and the relative performance of $\hat{\Theta}_3$ and $\hat{\Theta}_4$ should be more pronounced.

Discussion

The raw classification proportions estimator $\hat{\Theta}_1$ was shown to be inappropriate for composition estimation. Its bias can be considerable, e.g. for a 1971 brood year fishery consisting entirely of fish from the CENT stock group, the vector of raw classification proportions has expected value equal to the classification probabilities for fish from CENT. This is the third column of the above classification matrix, (0.1311, 0.1230, 0.6148, 0.1311). Since the true composition is $\Theta = (0,0,1,0)$, the bias of $\hat{\Theta}_1$ is (0.1311, 0.1230, −0.3852, 0.1311). This bias does not decrease with increased sample sizes and thus $\hat{\Theta}_1$ lacks the important statistical property of consistency and should not be used as an estimator of Θ.

The Cook and Lord (1978) corrected classification "estimator" $\hat{\Theta}_2$ permits nonsensical estimates of composition because elements of the estimated composition may be negative. Therefore it does not make sense to consider its statistical properties. The interesting comparisons are between estimators $\hat{\Theta}_3$, $\hat{\Theta}_4$, and $\hat{\Theta}_5$.

The simulations were not able to detect any clear difference in performance between the constrained corrected estimator $\hat{\Theta}_3$ and the maximum classification-likelihood estimator $\hat{\Theta}_4$. The direct maximum likelihood estimator $\hat{\Theta}_5$ was superior to $\hat{\Theta}_3$ and $\hat{\Theta}_4$.

The values in Tables 3 and 4 (means of the squared difference between estimated and true composition) allow for comparison of estimator performance, but they give little indication of the magnitude of the difference between the estimators at each simulation. Table 5 gives the average absolute difference between $\hat{\Theta}_3$ and $\hat{\Theta}_5$ for the simulations summarized in Tables 3 and 4. These average absolute differences range from 0.0125 (SEBC, 1977 brood year with 400 fish mixed sample) to 0.0411 (CENT, 1971 brood year with 100 fish mixed sample). The latter also produced the largest observed absolute difference, 0.1192.

It must be remembered that these simulations are not burdened by many of the difficulties encountered in practical composition estimation, e.g. measurement error, non-representative sampling. Different specification of sample sizes, number of variables used, and number of stock groups will also affect the relative performance of the estimators. However, I suspect that the direct maximum likelihood estimator $\hat{\Theta}_5$ will perform better than $\hat{\Theta}_3$ or $\hat{\Theta}_4$ except when there are very extreme

TABLE 5. The means of the absolute difference between Cook's (1983) constrained corrected classification estimator and the direct maximum likelihood estimator for the simulations summarized in Tables 3 and 4.

Regional stock group	1971 brood yr 100 fish mixed sample	1971 brood yr 400 fish mixed sample	1977 brood yr 100 fish mixed sample	1977 brood yr 400 fish mixed sample
ASIA	.0325	.0268	.0173	.0141
WEST	.0322	.0219	.0283	.0172
CENT	.0411	.0352	.0300	.0181
SEBC	.0352	.0271	.0181	.0125

violations to the model assumptions, in which case it is unlikely that any estimator will give useful results.

Should scientists who are currently using classification-based estimators switch to direct maximum likelihood? In models with a small number of stocks the classification estimators are convenient to calculate since $\hat{\Theta}_2$ will probably be non-negative and, if not, $\hat{\Theta}_3$ will be easy to calculate by hand. Thus, if the mixed fishery is only two or three stocks and particularly if the data are messy (see property 4 in the Theoretical Comparison of Estimators section) then there may be no advantage to using direct maximum likelihood. In other situations I feel that direct maximum likelihood can offer worthwhile improvements in performance.

Acknowledgements

For making this research possible, I thank Kate Myers, Colin Harris, Trey Walker, and Nancy Davis at the Fisheries Research Institute, University of Washington, Seattle. The manuscript was improved by comments from Chris Wood (Pacific Biological Station, Nanaimo, B.C., Canada). This work was partially supported by U.S. National Oceanic and Atmospheric Administration contract No. 50-ABNF-7-00002.

References

COOK, R. C. 1982. Stock identification of sockeye salmon (*Oncorhynchus nerka*) with scale pattern recognition. Can. J. Fish. Aquat. Sci. 39: 611–617.

1983. Simulation and application of stock composition estimators. Can. J. Fish. Aquat. Sci. 40: 2113–2118.

COOK, R. C., AND G. E. LORD. 1978. Identification of stocks of Bristol Bay sockeye salmon, *Oncorhynchus nerka*, by evaluating scale patterns with a polynomial discriminant method. Fish. Bull. 76: 415–423.

DEMPSTER, A. P., N. M. LAIRD, AND D. B. RUBIN. 1977. Maximum likelihood from incomplete data via the EM algorithm. J. R. Stat. Soc. B39: 1–38.

FOURNIER, D. A., T. D. BEACHAM, B. E. RIDDELL, AND C. A. BUSACK. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41: 400–408.

JOHNSON, R. A., AND D. W. WICHERN. 1982. Applied multivariate statistical analysis. Prentice-Hall, Englewood Cliffs, N.J. 594 p.

LACHENBRUCH, P. A. 1975. Discriminant analysis. Hafner Press, New York, NY. 128 p.

MILLAR, R. B. 1987. Maximum likelihood estimation of mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 44: 583–590.

1990. A versatile computer program for mixed stock fishery composition estimation. Can. Tech. Rep. Fish. Aquat. Sci. 1753: iii + 29 p.

MILNER, G. B., D. J. TEEL, AND F. M. UTTER. 1983. Genetic stock identification study. Annual report of research. NWAFC, NOAA, Seattle, WA. 98112.

MILNER, G. B., D. J. TEEL, F. M. UTTER, AND C. L. BURLEY. 1981. Columbia River stock identification study: validation of genetic method. Annual report of research (FY80). NWAFC, NOAA, Seattle, WA. 98112.

MULLIGAN, T. J., S. MCKINNELL, AND C. C. WOOD. 1988. Uncertainty in stock composition estimates of oceanic steelhead trout using electrophoretic characteristics: comments on a recent study. Can. J. Fish. Aquat. Sci. 45: 432–442.

MYERS, K. W., D. E ROGERS, C. K. HARRIS, C M. KNUDSEN, R. V. WALKER, AND N. D. DAVIS. 1984. Origins of chinook salmon in the area of the Japanese mothership and landbased driftnet salmon fisheries in 1975–1981. (Document submitted to annual meeting of the International North Pacific Fisheries Commission, Vancouver, Canada, November 1984.) 208 p. Fisheries Research Institute, University of Washington, Seattle.

OKAZAKI, T. 1985. Distribution and migration of *Salmo gairdneri* and *Salmo mykiss* in the North Pacific based on allelic variation of enzymes. Jpn. J. Ichthyol. 32: 203–215.

PELLA, J. J., AND G. B. MILNER. 1987. Use of genetic marks in stock composition analysis, p. 247–276. *In* N. Ryman and F. M. Utter [ed.] Population genetics and applications to fisheries management. University of Washington Press, Seattle, WA.

PELLA, J. J., AND T. L. ROBERTSON. 1979. Assessment of composition of stock mixtures. Fish. Bull. 77: 387–398.

WOOD, C. C., S. MCKINNELL, T. J. MULLIGAN, AND D. A. FOURNIER. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. 44: 866–881.

WORLUND, D. D., AND R. A. FREDIN. 1962. Differentiation of stocks, p. 143–153. *In* Symposium on pink salmon. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C.

WU, C. F. J. 1983. On the convergence properties of the EM algorithm. Ann. Stat. 11: 95–103.

Changes in the Composition of Snow Crab (*Chionoecetes opilio*) Participating in the Annual Breeding Migration in Bonne Bay, Newfoundland

G. P. Ennis

Science Branch, Department of Fisheries and Oceans, P. O. Box 5667, St. John's, Newfoundland A1C 5X1 Canada

R. G. Hooper

NICOS/Biology Department, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9 Canada

and D. M. Taylor

Science Branch, Department of Fisheries and Oceans, P. O. Box 5667, St. John's, Newfoundland A1C 5X1 Canada

Ennis, G. P., R. G. Hooper, and D. M. Taylor. 1990. Changes in the composition of snow crab (*Chionoecetes opilio*) participating in the annual breeding migration in Bonne Bay, Newfoundland. Can. J. Fish. Aquat. Sci. 47: 2242–2249.

Sexually-paired snow crabs (*Chionoecetes opilio*) participating in an annual spring breeding migration to shallow water in Bonne Bay, Newfoundland were collected by SCUBA divers from 1984 to 1989. In 1984 the breeding pairs were composed mainly of relatively large, old-shell animals of both sexes, but by 1988 much smaller animals, most of which were new-shell, dominated the migration. Evidence from research fishing indicates that these changes are related to a rapid decline in what was characteristically a virgin population in 1984 and a coinciding strong pulse of recruitment into the breeding component of the population. A very high incidence of small, subcommercial males participating in the 1988 and 1989 breeding migrations demonstrates a capacity for maintaining a high level of reproductive potential in a snow crab population despite high exploitation rates on larger animals in the male-only fishery for this species. The strong recruitment pulse which occurred in Bonne Bay as the virgin population declined after 1984 also indicates a capacity for enhanced annual production within a snow crab population when exploitation rates are high.

Des couples de crabes des neiges (*Chionoecetes opilio*) participant à une migration printanière annuelle de reproduction vers les eaux peu profondes de Bonne Bay (Terre-Neuve) ont été recueillis par des scaphandriers de 1984 à 1989. En 1984, les couples reproducteurs étaient formés principalement d'animaux des deux sexes relativement gros et à coquille ancienne alors que, en 1988, la majorité des crabes en migration étaient beaucoup plus petits et la plupart d'entre eux avaient une nouvelle coquille. Les résultats de la pêche aux fins de recherche indiquent que ces changements sont liés à un rapide déclin dans ce qui était une population non exploitée typique en 1984 et qu'ils coïncident avec un fort recrutement du groupe reproducteur de cette population. Une incidence très élevée de petits mâles de taille non commerciale, ayant participé aux migrations de reproduction 1988 et 1989, démontre la capacité de maintenir un niveau élevé de potentiel de reproduction chez une population de crabes des neiges malgré de forts taux d'exploitation des plus gros animaux, pour la pêche axée exclusivement sur les mâles de cette espèce. Le fort recrutement survenu à Bonne Bay au moment où la population non exploitée baissait après 1984 indique en outre une capacité d'amélioration de la production annuelle chez une population de crabes des neiges soumise à des taux d'exploitation élevés.

Received March 10, 1989
Accepted May 10, 1990
(JA086)

Reçu le 10 mars 1989
Accepté le 10 mai 1990

During April–May each year, sexually-paired snow crabs, *Chionoecetes opilio*, migrate into shallow water (10–30 m) in Bonne Bay, Newfoundland, from depths possibly as great as 150 m. Various behavioral, ecological, and biological aspects of this breeding migration were described by Taylor et al. (1985) and Hooper (1986) based on SCUBA diver observations and sampling in 1982, 1983, and 1984. There has been no legal snow crab fishery in the Bay and the population was considered to be virgin in 1984 (Taylor et al. 1985). Typically, pairs included a relatively large, old-shell male and a much smaller, old-shell female. Although males reach sexual maturity over the 51–72 mm carapace width (CW) size range (Watson 1970), 304 paired males collected during the breeding migration in 1984 ranged from 89 to 140 mm CW. Keen competition between single and paired males for possession of females (Hooper 1986) probably eliminates small, mature males from participating in breeding activity in an unexploited population. All paired females collected in 1984 were multiparous (females that have produced more than one egg clutch), each had recently hatched a clutch of eggs, was carrying, or in the process of hatching well-developed eggs, and each also had a ripe ovary indicating extrusion of a new clutch of eggs to be imminent. These females ranged in size from 55 to 86 mm CW (Taylor et al. 1985).

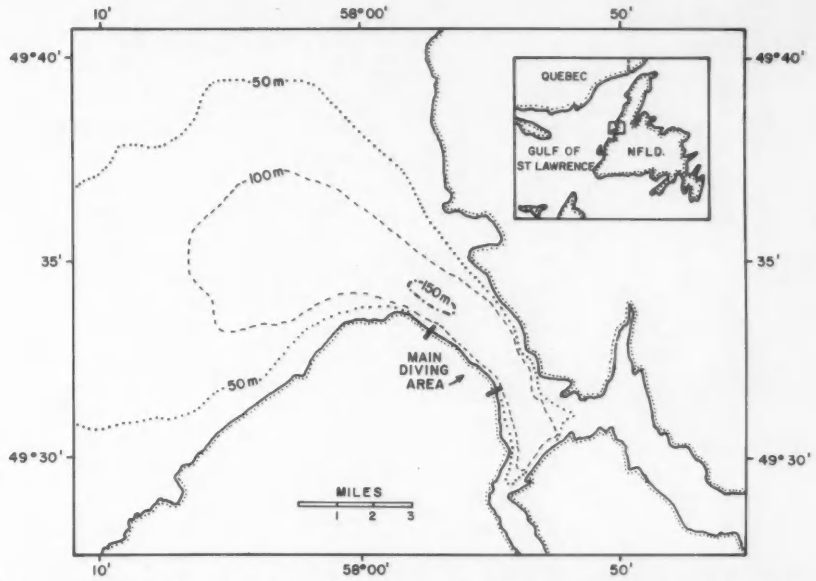

FIG. 1. Map of the outer portion of Bonne Bay, Newfoundland, with depth contours and indicating the general area where collection of sexually-paired snow crabs *Chionoecetes opilio* was concentrated.

TABLE 1. Carapace width and shell condition of sexually-paired male and female *Chionoecetes opilio* collected by SCUBA divers in Bonne Bay, Newfoundland, 1984–89.

		Males				Females		
Year	N	$\overline{CW}$ (mm)	Range	% <95 mm	% new-shell[a]	$\overline{CW}$ (mm)	Range	% new-shell
1984	304	116.6	89–140	1.0	16.4	70.0	55–86	3.3
1985	199	113.8	76–132	2.0	7.0	69.9	60–90	1.0
1986	15	111.1	94–123	6.7	0.0	69.1	64–82	0.0
1987	31	100.3	62–126	32.3	12.9	69.9	58–82	0.0
1988	208	82.7	61–133	87.5	80.0	63.8	49–78	79.3
1989	264	82.3	65–111	88.6	76.1	64.0	50–79	72.0

[a]No soft-shell crabs (i.e. very recent molters) were observed in these samples. Observations on recaptured crabs tagged in soft-shell condition on the northeast coast of Newfoundland indicate that what we refer to here as new-shell crab may have molted as recently as 4 mo (Taylor et al. 1989) but not more than 2 yr (D. M. Taylor, unpubl. data) previous.

Snow crabs in Bonne Bay appear to be isolated geographically from populations elsewhere in the Gulf of St. Lawrence. A shallow sill near the mouth of the Bay (Fig. 1) would tend to restrict movements in or out and there is no known concentration in the Gulf near Bonne Bay. After 1984, an illegal fishery on Bonne Bay snow crab developed rapidly and this reduced the abundance of commercial size (≥95 mm CW) males (Ennis et al. 1988a). In 1986, and particularly in 1987, there were substantial increases in the percentage of males <95 mm CW (32.3% in 1987 cf. 1.0% in 1984) participating in pairing presumably because of reduced competition for possession of females (Ennis et al. 1988b). Among paired females collected from 1983 to 1987, there were no changes in size composition or reproductive status (Ennis et al. 1988b; G. P. Ennis, unpubl. data).

Sampling during the breeding migration in 1988 and again in 1989 revealed further, much more substantive changes in the composition of paired males and also equally substantive changes among the females. Our purpose is to describe these changes and consider their relevance to current issues in management of snow crab fisheries in Atlantic Canada.

Materials and Methods

Sexually-paired snow crabs were collected by SCUBA divers at depths from 10 to 30 m during the annual (April–May) breeding migration to shallow water in Bonne Bay each year from 1984 to 1989. In most cases, each pair was kept in a separate mesh bag. While mating crabs were present in shallow water, samples were also obtained from depths >90 m using small-mesh traps from 1985 to 1989. This research fishing was carried out near the area along the south shore of Bonne Bay where diver sampling was concentrated (Fig. 1). Maximum CW of each crab was measured to the nearest 1.0 mm and shell condition was classified, according to the criteria of Miller and O'Keefe (1981) and Taylor et al. (1989) as new or old. Chela height was measured to the nearest 0.1 mm for diver-caught, paired males each year starting in 1986 and for trap-caught

TABLE 2. Carapace width and shell condition of male and female *Chionoecetes opilio* caught in research fishing with small-mesh traps at depths >90 m in Bonne Bay, Newfoundland during May–June, 1988 and 1989.

		Males				Females			
Year	N	$\overline{CW}$ (mm)	Range	% <95 mm	% new-shell[a]	N	$\overline{CW}$ (mm)	Range	% new-shell
1988	221	85.8	58–136	72.9	73.3	148	70.3	54–85	20.3
1989	533	83.4	48–133	75.6	61.4	132	70.4	54–84	12.9

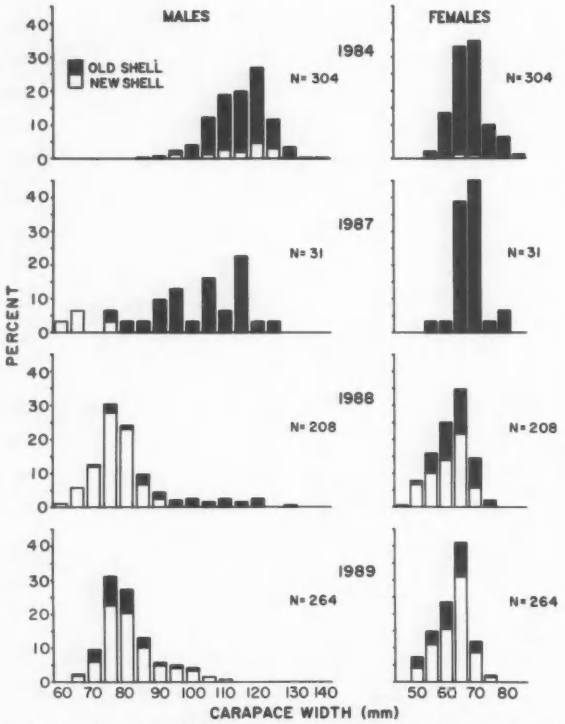

FIG. 2. Size composition and shell condition of diver-caught, sexually-paired male and female *Chionoecetes opilio* from Bonne Bay, Newfoundland 1984 and 1987–89.

males in 1988 and 1989. A carapace width-chela height equation was derived from discriminant analysis of data from the 1988 trap-caught sample. This provides a basis for distinguishing between morphometrically mature and immature males (as per Conan and Comeau 1986) in Bonne Bay samples. The egg masses of females were examined and categorized as new (orange with no eye spots) or old (well developed with conspicuous eye spots). The extent of hatching was approximated and for those in which hatching had been completed, the presence of newly-extruded (bright orange) eggs was noted.

While searching for paired crabs in early May 1989, divers found numerous cast shells of molted crabs at depths of 20–35 m. Specimens were collected and kept individually in mesh bags. These were sexed and carapace width and chela height measured as accurately as possible.

Results

Composition of Sexually Paired Males and Females

The composition of sexual pairs collected during the annual breeding migration in Bonne Bay changed dramatically from 1984 to 1988 (Table 1, Fig. 2). The mean size of males decreased significantly from 116.6 mm CW in 1984 to 100.3 mm in 1987 (Tukey test, $P < 0.001$) and to 82.7 mm in 1988 ($P < 0.001$) and remained unchanged ($P > 0.5$) at 82.3 mm in 1989. The percentage of paired males <95 mm CW increased significantly from 1% in 1984 to 32.3% in 1987 ($P < 0.001$) and to 87.5% in 1988 ($P < 0.001$) and was nearly the same ($P > 0.5$) at 88.6% in 1989. For females, there was no change ($P > 0.5$) in the mean size from 1984 to 1987 (70.0 and 69.9 mm CW, respectively) but it decreased significantly ($P < 0.001$) to 63.8 mm in 1988 and remained unchanged ($P > 0.5$) at 64.0 mm in 1989.

From 1984 to 1987, 12.4% and 2.2% of the paired males and females, respectively, were new-shell, but in 1988 80.0% of the males and 79.3% of the females and in 1989 76.1% and 72.0%, respectively, were new-shell (Table 1). In 69.2% of the pairs collected in 1988 both the male and female were new-shell and in 10.1% both were old-shell; these percentages were 55.3 and 7.2, respectively, in 1989.

The very substantial shift in the composition of paired crabs participating in the breeding migration in Bonne Bay over a 4–5 yr period indicates a capacity for dynamic population responses in this species. The tremendous increase in the incidence of subcommercial (<95 mm CW) males participating in the 1988 and 1989 migrations demonstrates a considerable capacity within a snow crab population to maintain reproductive potential at a high level despite a high level of annual removals of larger animals in the male-only fishery.

TABLE 3. Comparison of shell condition and egg development of diver-caught and trap-caught female *Chionoecetes opilio* in Bonne Bay, Newfoundland, May–June 1988 and 1989.

	Old-shell			New-shell		
	N	New eggs	Old/hatching eggs	N	New eggs	Old/hatching eggs
1988						
Diver-caught	43	0	43	165	0	165
Trap-caught	118	116	2	30	10	20
1989						
Diver-caught	74	0	74	190	1	189
Trap-caught	115	69	46	17	16	1

Comparison of 1988 and 1989 Diver- and Trap-Caught Samples

The mean size of males in trap-caught samples from depths >90 m during May–June, 1988 was 85.8 mm CW, slightly larger than the 82.7 mm CW of the diver-caught males (Table 1, 2). Among trap-caught males the percentage <95 mm CW was lower (72.9% cf. 87.5%) and the percentage new-shell was

TABLE 4. Summary of snow crab, *Chionoecetes opilio*, catches in research fishing with small-mesh traps at depths >90 m in Bonne Bay, Newfoundland during May–June, 1985–89.

	Males ≥95 mm CW			Males <95 mm CW			Females[b]		
Year	N	CPUE[a]	% new-shell	N	CPUE[a]	% new-shell	N	CPUE[a]	% new-shell
1985	260	23.6	8.1	19	1.7	15.8	464	38.7	0.0
1986	355	12.2	3.9	45	1.6	15.6	546	18.8	0.9
1987	19	3.8	31.6	145	29.0	94.5	35	7.0	51.4
1988	60	8.6	15.0	161	21.7	95.4	148	22.4	24.2
1989	130	4.6	68.5	403	14.4	59.1	132	4.7	12.9

[a]Number per trap haul.
[b]Immature females are excluded.

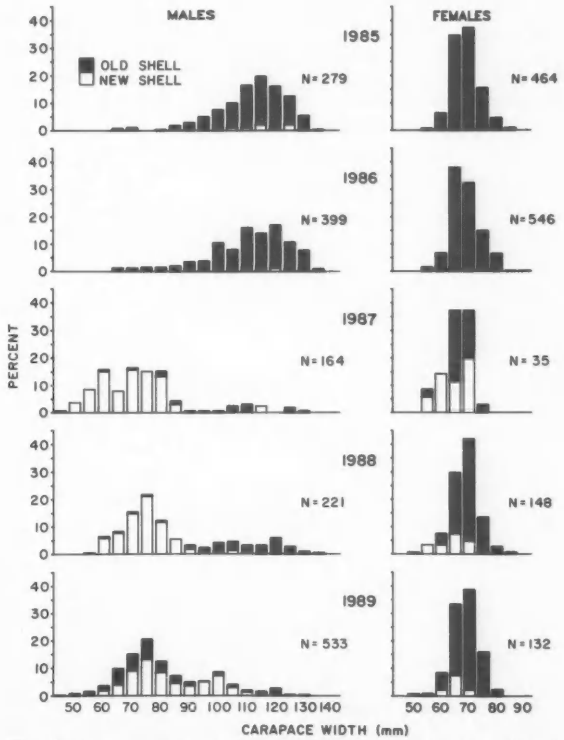

FIG. 3. Size composition and shell condition of trap-caught male and female *Chionoecetes opilio* from Bonne Bay, Newfoundland during May–June, 1985–89.

also lower (73.3% cf. 80%) compared to the diver-caught males. There was also relatively little difference in the composition of males between the 1989 diver- and trap-caught samples and for both it was very similar to the 1988 samples (Table 1, 2). Assuming no selection bias which would invalidate the comparison, these observations indicate little difference in the composition of males participating in the breeding migration to shallow water and those in adjacent deep water at around the same time.

There were substantial differences, however, in the composition of females in these diver-caught and trap-caught samples. The 1988 diver-caught females were smaller than the trap-caught (CW = 63.8 mm cf. 70.3 mm; *t*-test, $P < 0.001$) and mostly new-shell (79.3% cf. 20.3%) (Table 1, 2). As with the males, there was relatively little difference in size and shell-condition composition between 1988 and 1989 for either the diver- or trap-caught samples (Table 1, 2).

In addition, egg development differed between diver- and trap-caught females as well as between the 1988 and 1989 trap-caught females (Table 3). All of the diver-caught females (both old- and new-shell) were carrying old, eyed eggs or were in the process of hatching or had very recently completed hatching their eggs. In the 1989 sample, one of these females was carrying a clutch of very recently extruded, bright orange eggs. It presumably had hatched its old eggs earlier. In contrast, most of the trap-caught females (85.1% in 1988 and 64.4% in 1989) were carrying new (orange) eggs, which likely had been extruded sometime within the preceding 2–3 mo, the remainder were carrying old eggs similar to those of the diver-caught females (Table 3). Among old-shell females, the percentage with orange eggs decreased from 98.3% in 1988 to 60.0% in 1989 and among new-shell females it increased from 33.3% in 1988 to 94.1% in 1989 (Table 3).

The high incidence of relatively new-egged females in deep water in contrast to the old-egged, sexually-paired females found in shallow water at around the same time indicates that substantial breeding activity occurs within the population prior to and independent of the shallow-water migration each spring.

Changes in Relative Abundance, 1985–89

Although research trap fishing in deep water at the same time that paired crabs were present in shallow water was not extensive, the summarized catches provide a qualitative indication of changes that occurred in the Bonne Bay population from 1985 to 1989 (Table 4, Fig. 3). Of particular relevance here are the changes from 1985 to 1987. Catch rates of male crabs ≥95 mm CW in May–June research fishing dropped from 23.6 per trap haul in 1985 to 3.8 in 1987, a substantial decline (*t*-test, $P = 0.01$) especially considering that the percentage new-shell increased from 8.1 to 31.6% (Table 4). Between 1985 and 1987, catch rates of males <95 increased from 1.7 to 29.0 per trap haul (*t*-test, $P = 0.11$; a Mann–Whitney test gave $P < 0.01$) and the percentage new-shell increased from 15.8 to 94.5% (Table 4). There was also a substantial decline (*t*-test, $P < 0.01$) in the catch rate of females from 38.7 per trap haul, all of which were old-shell, in 1985 to 7.0 per trap haul, of which 51.4% were new-shell, in 1987 (Table 4).

Changes in catch rates and shell condition since 1987 have been inconsistent between groups. The catch rate of males ≥95 mm increased slightly in 1988 but dropped again in 1989 and percentage new shell dropped in 1988 but increased to 68.5%, which was more than double the previous high, in 1989 (Table

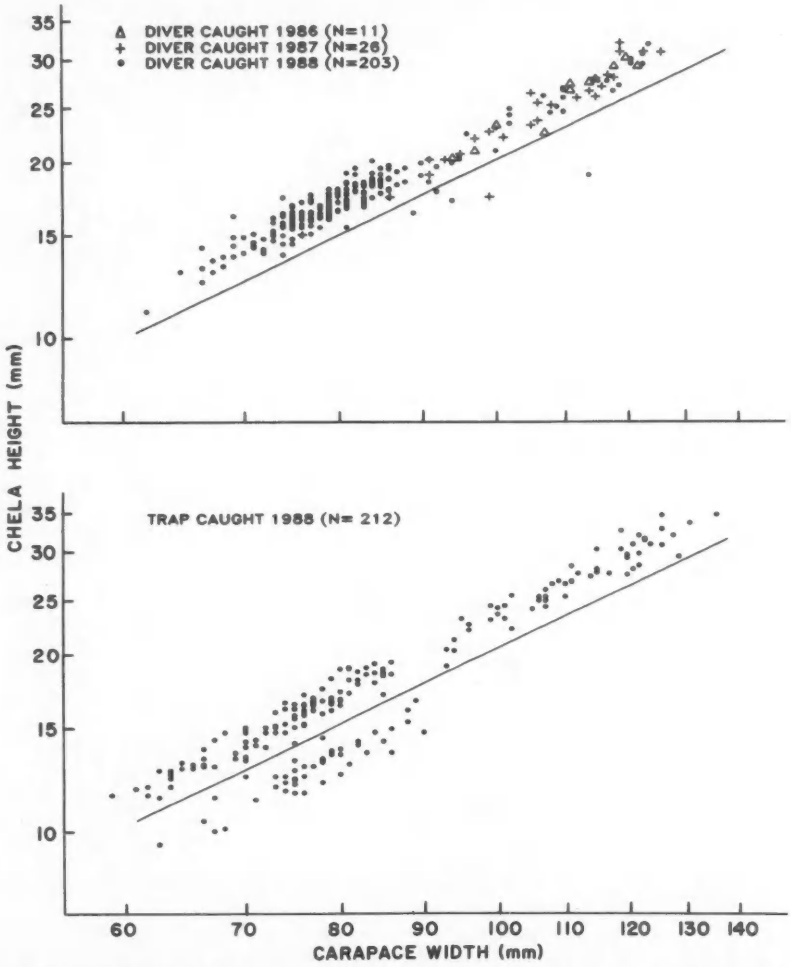

FIG. 4. Natural log-log plots of carapace width vs. chela height for the 1986, 1987, and 1988 diver-caught and the May–June 1988 trap-caught male *Chionoecetes opilio* in Bonne Bay, Newfoundland. The cutting line (l_n CH = 1.3330 l_n CW − 3.1281) is from discriminant analysis of the 1988 trap-caught sample: crabs above the line are morphometrically mature.

4). For males <95 mm catch rates dropped in 1988 and in 1989; percentage new shell remained very high in 1988 and dropped in 1989, but for both, the 1989 values were much higher than observed prior to 1987. The catch rate of females increased substantially in 1988 but dropped in 1989 to the lowest value observed. Percentage of new-shell females dropped each year from the high value observed in 1987.

Catches in research trap fishing indicate that the apparently virgin snow crab population present in Bonne Bay in 1984 declined rapidly to a low level of abundance by 1987. A strong pulse of newly-recruited animals, which largely replaced the virgin population, became evident in the 1987 catches.

Morphometric Maturity

The line from the equation l_n CH = 1.3330 l_n CW − 3.1281, which was derived from discriminant analysis of the carapace-width/chela-height data from the 1988 trap-caught sample of males, was superimposed on plots of the data for various samples to distinguish between morphometrically mature and immature males. About 2% of the paired males collected during the 1986–1988 breeding migrations were categorized as morphometrically immature (i.e. below the line) and in 1989 it was up to around 10% (Fig. 4, 5). In 1988, there were no morphometrically immature males smaller than 89 mm CW in the diver-caught pairs and none larger than 90 mm in the trap-caught sample (Fig. 4). However, in 1989 most of the morphometrically immature males in the diver-caught pairs were smaller than 89 mm (the smallest was 68 mm) and in the trap-caught sample there were several larger than 90 mm (the largest was 116 mm) (Fig. 5). These observations demonstrate that male crabs from at least 70–114 mm CW which can be readily categorized as morphometrically immature can and do participate in mating activity in the wild despite being at a compet-

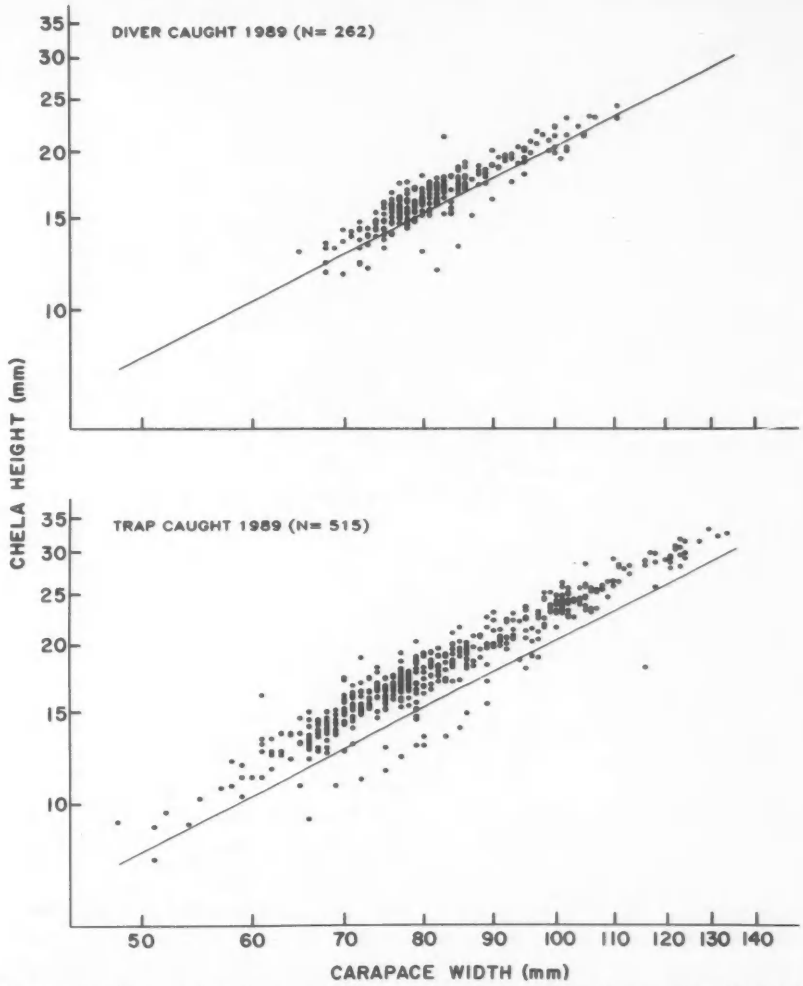

FIG. 5. Natural log-log plots of carapace width vs. chela height for the 1989 diver-caught and the May–June 1989 trap-caught male *Chionoecetes opilio* in Bonne Bay, Newfoundland. The cutting line (l_n CH = 1.3330 l_n CW − 3.1281) is from discriminant analysis of the 1988 trap-caught sample.

itive disadvantage with morphometrically mature males of the same size.

In early May 1989, before any paired crabs had been found in shallow water, numerous cast shells of molted crabs were seen in depths of around 20–35 m. The only live crabs seen in the area at the time were two soft-shell animals. The cast shells were frail and pliable making accurate carapace width and chela height measurements difficult. However, the data indicate that participation in the mass movement to shallow water for molting was restricted to morphometrically immature males from 53 to 104 mm CW (Fig. 6). Recent molting would likely preclude participation by males in mating and this may also account for the relative scarcity of morphometrically immature males participating in the breeding migration.

Discussion

From 1984 to 1988 there was a rapid and very substantial shift in the composition of sexually-paired snow crabs participating in the annual spring breeding migration to shallow water in Bonne Bay. In 1984, the breeding migration was dominated by large, old-shell individuals, but in 1988 it was dominated by smaller, new-shell animals. The amount of diver-collecting effort was similar each year so the smaller number of pairs collected in 1986 and 1987 reflects a much reduced abundance of pairs in shallow water than in 1984 and 1985. There was a significant shift to smaller males in 1987 but the major shift to even smaller males, significantly smaller females and predominantly new-shell animals occurred in 1988 when the abundance of pairs increased to a level comparable to 1984 and 1985. In 1989, the abundance of pairs and their composition was similar to 1988. Evidence from research trap fishing in deep water (>90 m) from 1985 indicates that the changes observed in the sexually-paired crabs collected in shallow water each spring were due to a very rapid decline in what was characteristically a virgin population in 1984 and a coinciding strong

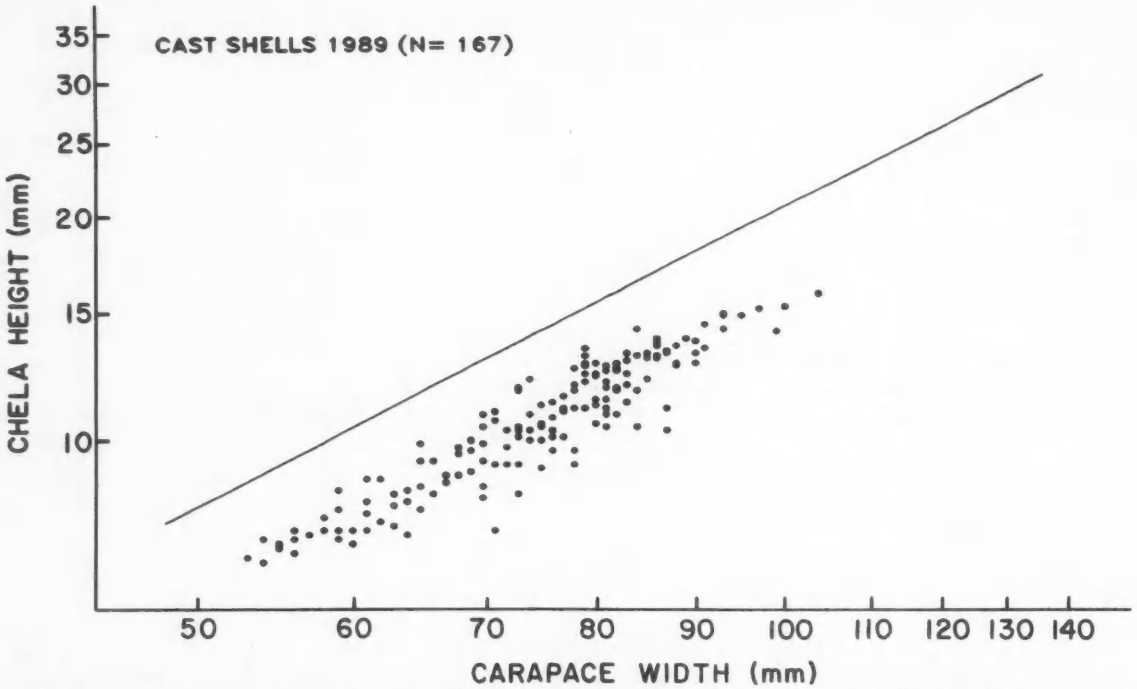

FIG. 6. Natural log-log plot of carapace width vs. chela height for cast shells of male *Chionoecetes opilio* collected by SCUBA divers at depths of 20–35 m in Bonne Bay, Newfoundland in May 1989. The cutting line (l_n CH = 1.3330 l_n CW − 3.1281) is from discriminant analysis of the 1988 trap-caught sample.

pulse of recruitment that largely resulted in a new breeding population.

There has been no legal snow crab fishery in Bonne Bay and our evidence that an illegal fishery developed rapidly after 1984 is anecdotal. From extensive contact with Bonne Bay residents there is no question that poaching of snow crabs became quite prevalent during the period in question. Gill nets were used extensively in this fishery and these catch females, which are unattractive for harvest because of their small size and negligible meat yield, as well as the larger males. Females were likely destroyed because of the difficulty of removing them from the gill nets intact. The Bonne Bay snow crab population occupies a relatively small area and it is quite feasible that poaching could have a significant impact. Also, since 1983 research sampling of snow crabs in Bonne Bay has been extensive and may have precipitated the poaching activity, contributing significantly to total removals from the population. The foregoing appears to be the most plausible explanation for the rapid decline, as indicated by our research trap fishing as well as diver sampling, in the population that was present in 1984.

The animals which formed the pulse of recruitment to the breeding component of the population in 1988 were probably present in the population as very small juvenile crabs in 1984. It seems reasonable to suggest that conditions for their growth and survival were much improved by the reduced abundance of large males especially, after 1984. This might have been an important factor in determining the relative strength of the recruitment pulse which became so evident in the trap-caught samples in 1987 and in the diver-caught samples in 1988.

Ennis et al. (1988b) considered the significantly increased incidence of males <95 mm CW (the minimum legal size in the snow crab fishery in Atlantic Canada) participating in the 1987 breeding migration in Bonne Bay as evidence that, contrary to the conclusion by Conan and Comeau (1986), sublegal males can mate in nature with multiparous females. The further substantial shift to small males (some as small as 61 mm CW) in the 1988 and 1989 migrations clearly demonstrates that sublegal males are capable of participating fully in natural mating activity.

Conan and Comeau (1986) concluded that morphometrically immature males do not participate in mating activity regardless of size. Among the sexually-paired males we collected in Bonne Bay from 1986 to 1989, there were many ranging from around 70 to 114 mm CW that could be categorized as morphometrically immature. Our observations indicate that a mass molting event which involved only morphometrically immature males took place in Bonne Bay only weeks before the 1989 breeding migration. This is the first time that evidence of such a phenomenon has been observed in Bonne Bay. Recent molting by a large proportion of morphometrically immature males in the population may explain their relative scarcity in the Bonne Bay breeding migration, but our observations show that they do participate in mating in the wild.

The spring breeding migration to shallow water, which involved only females that are about to hatch old eggs and extrude a new clutch, is just one component of the overall annual reproductive cycle in the Bonne Bay snow crab population. The presence in deep water at around the same time of large numbers of females, both old- and new-shell, with rela-

tively new eggs demonstrates that substantial breeding activity occurs prior to the shallow-water migration. Spawning and presumably subsequent hatching occurs over a more protracted period than indicated by the breeding migration alone. A long hatching period may enhance population reproductive success.

We have documented very dynamic changes that occurred in the Bonne Bay snow crab population between 1984 and 1988. Our observations demonstrate that sublegal males can maintain a high level of reproductive potential in a snow crab population if exploitation rates on commercial-size males are high. That the recruitment pulse in Bonne Bay coincided with a rapid decline in the virgin population is also suggestive of a capacity for enhanced productivity (i.e. annual production of commercial biomass) in a snow crab population, associated with improved conditions for growth and survival of smaller crabs, when exploitation rates are high.

Acknowledgments

We are indebted to D. G. Badcock for assistance in the field, particularly with diving, to P. G. O'Keefe for assistance in the field and with laboratory processing of samples and data, and to P. W. Collins for assistance with data analyses.

References

CONAN, G. Y., AND M. COMEAU. 1986. Functional maturity and terminal molt of male snow crab, *Chionoecetes opilio*. Can. J. Fish. Aquat. Sci. 43: 1710–1719.

ENNIS, G. P., R. G. HOOPER, AND D. M. TAYLOR. 1988a. Changes in size composition of male crabs (*Chionoecetes opilio*) participating in the annual breeding migration in Bonne Bay, Newfoundland. Can. Atl. Fish. Sci. Adv. Comm. Res. Doc. 88/2: 14 p.

1988b. Functional maturity in small male snow crabs (*Chionoecetes opilio*). Can. J. Fish. Aquat. Sci. 45: 2106–2109.

HOOPER, R. G. 1986. A spring breeding migration of the snow crab, *Chionoecetes opilio* (O. Fabr.) into shallow water in Newfoundland. Crustaceana 50: 257–264.

MILLER, R. J., AND P. G. O'KEEFE. 1981. Seasonal and depth distribution, size, and molt cycle of the spider crabs, *Chionoecetes opilio*, *Hyas araneus* and *Hyas coarctatus* in a Newfoundland Bay. Can. Tech. Rep. Fish. Aquat. Sci. 1003: iv + 18 p.

TAYLOR, D. M., R. G. HOOPER, AND G. P. ENNIS. 1985. Biological aspects of the spring breeding migration of snow crabs, *Chionoecetes opilio*, in Bonne Bay, Newfoundland (Canada). Fish. Bull. U.S. 83: 707–711.

TAYLOR, D. M., G. W. MARSHALL, AND P. G. O'KEEFE. 1989. Shell hardening in snow crab tagged in soft-shell condition. N. Am. J. Fish. Manage. 9: 504–508.

WATSON, J. 1970. Maturity, mating and egg laying in the spider crab, *Chionoecetes opilio*. J. Fish. Res. Board Can. 27: 1607–1616.

Genetic Differences in the Early Development and Growth of Sympatric Sockeye Salmon and Kokanee (*Oncorhynchus nerka*), and their Hybrids

Chris C. Wood and Chris J. Foote[1]

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, British Columbia V9R 5K6 Canada

Wood, C. C., and C. J. Foote. 1990. Genetic differences in the early development and growth of sympatric sockeye salmon and kokanee (*Oncorhynchus nerka*), and their hybrids. Can. J. Fish. Aquat. Sci. 47: 2250–2260.

Anadromous (sockeye salmon) and nonanadromous (kokanee) *Oncorhynchus nerka* spawn sympatrically yet appear genetically distinct in a number of rivers in British Columbia. To investigate whether genetic differences are maintained by selection against "hybrid" progeny, we raised pure and reciprocal crosses of Shuswap River sockeye and kokanee under controlled hatchery conditions. Sockeye eggs were larger and survived slightly better than kokanee eggs, regardless of male type, both to the eyed egg stage and as young fry. We observed no differences in survival among cross types during the remainder of the 460 d study. Rate of yolk absorption was similar in pure sockeye and pure kokanee alevins, but significantly faster in alevins sired by sockeye than those sired by kokanee. This indicates a male genetic effect which compensates for the difference in egg size. Hybrid alevins developed differently because egg size is mismatched with the male genotype. Growth rates of fry were significantly more variable within pure kokanee families than within pure sockeye families. Hybrid crosses survived as well as pure crosses under the study conditions. However, any progeny resulting from hybrid crosses in nature may sustain higher mortality than those from pure crosses.

Les formes anadromes et non-anadromes de *Oncorhynchus nerka* frayent simultanément mais semblent pourtant génétiquement distinctes dans un certain nombre de cours d'eau de Colombie-Britannique. Afin de vérifier si ces différences génétiques sont le produit d'une sélection s'opérant au détriment de la progéniture "hybride", nous avons élevé des croisements purs et réciproques de formes anadromes et non-anadromes de saumons de la rivière Shuswap en conditions contrôlées. Les oeufs des formes anadromes sont plus gros et survivent légèrement mieux que ceux des formes non-anadromes, quel que soit le type de mâle, aussi bien au stade embryonné qu'à celui de jeune alevin. Nous n'avons pas noté de différence dans les taux de survie entre les types de croisement durant le reste de l'étude. Le taux d'absorption du vitellus était similaire chez les alevins d'anadrome pur et de non-anadrome pur mais notablement plus rapide chez les alevins des croisements produits par un anadrome mâle que chez les alevins des croisements produits par un non-anadrome mâle. Cela révèle un effet génétique mâle qui compense la différence de taille des oeufs. Les alevins hybrides se sont développés différemment parce que la taille des oeufs n'est pas en rapport avec le génotype mâle. Les taux de croissance des alevins fluctuaient bien davantage au sein des familles de non-anadromes purs qu'au sein des familles d'anadromes purs. Les croisements hybrides ont aussi bien survécu que les croisements purs dans les conditions expérimentales. Toutefois, dans la nature, toute progéniture de croisements hybrides pourrait être affectée d'une mortalité supérieure à celle des croisements purs.

Received January 8, 1990
Accepted June 1, 1990
(JA434)

Reçu le 8 janvier 1990
Accepté le 1 juin 1990

The possibility of genetic divergence of sympatric forms of the same species has been demonstrated in numerous theoretical models (Maynard Smith 1966; Rosenzweig 1978; Pimm 1979; Rice 1984). In theory, divergence of sympatric forms, and ultimately speciation, can result from disruptive selection against the offspring of hybrid crosses between the forms (post-zygotic isolating mechanisms), especially where the forms tend to mate assortatively, by size, for example (pre-zygotic isolating mechanisms) (Rice 1984). However, sympatric genetic divergence is difficult to observe in nature and the significance of its role in speciation is widely disputed (Mayr 1970; Futuyma and Mayer 1980; Templeton 1981; but see West–Eberhard 1989).

The Pacific salmon *Oncorhynchus nerka* occurs as two types: the anadromous sockeye salmon and the nonanadromous kokanee. Kokanee mature at smaller sizes and often at younger ages than sockeye, and where the two forms occur together, they sometimes exhibit other morphological differences (e.g. gill raker number, male secondary sexual characteristics, and colour) (Nelson 1968; Ricker 1940). The pattern of genetic differentiation among sockeye and kokanee populations in British Columbia suggests that the two types have diverged in sympatry on numerous, independent occasions (Foote et al. 1989b). In some lakes, the two types remain sympatric and spawn in the same locations at the same time (Ricker 1940; Hanson and Smith 1967; McCart 1970; Foote 1987; Foote and Larkin 1988). Where this occurs, there is a high degree of assortative mating by size, and hence by type (Foote and Larkin 1988). However,

[1]Present address: Department of Biology, McGill University, 1205 Docteur Penfield Ave., Montreal, Quebec H3A 1B1 Canada.

the types do attempt to interbreed — kokanee males commonly "sneak in" on spawning sockeye pairs (Hanson and Smith 1967; McCart 1970; Foote and Larkin 1988). Despite obvious opportunities for gene flow to occur, the types appear genetically distinct (using protein electrophoretic techniques) where they spawn sympatrically (Foote et al. 1989b).

The observed biochemical genetic differences between sympatric sockeye and kokanee could not persist if the two types interbreed to the extent suggested unless: (1) the biochemical evidence for genetic differentiation is an artifact of continual natural selection against particular alleles examined, perhaps associated with the physiological requirements of anadromy; (2) kokanee males are unsuccessful in their sneaking attempts to fertilize sockeye eggs; or (3) there is strong, natural selection against hybrid progeny of the two types. It seems improbable that selection acting directly on the allozymes examined could account for the repeated differences reported by Foote et al. (1989b). Significant differences in allele frequency were evident at more than one locus within all three lakes surveyed, and the loci in question differed between the Skeena and Fraser river drainages. Hence, to save the first explanation, one must postulate that selection acts at four different loci. Also, it seems almost certain that the sneaking behaviour of kokanee males can lead to the successful fertilization of sockeye eggs. Sneaking behaviour has been shown to be successful in four independent studies of other salmonids (Schroder 1982; Chebanov et al. 1983; Maekawa and Onozato 1986; Hutchings and Myers 1988); moreover, the authors have demonstrated experimentally that sneaking kokanee males can successfully fertilize sockeye eggs in Pierre Creek, Babine Lake (C. Foote and C. Wood, unpubl. data). Sockeye eggs fertilized artifically with kokanee milt also produce viable hybrid fry (McCart 1970; this study).

The simplest hypothesis to account for genetic differentiation between sympatric sockeye and kokanee is that the fitness of hybrids is less than that of pure types. In this paper, we investigate potential mechanisms by which disruptive selection could maintain genetic differences between the two types. We looked for differences between the pure and hybrid crosses of kokanee and sockeye with respect to fertilization success, incubation times, and rates of survival and growth in a controlled hatchery environment during their first year while both types would typically inhabit the same freshwater environment.

Methods

Broodstock Collection

Sockeye and kokanee spawn at the same time and in the same or adjacent locations within the Lower Shuswap River, British Columbia, yet appear to be genetically distinct populations (Foote et al. 1989b). Random samples of ripe sockeye and kokanee were collected with small seine nets on October 12, 1986 from which 9 males and 18 females of each type were selected for their readiness to spawn. Postorbital-hypural length measurements were recorded and scales and otoliths were removed for age determination. Age was determined from scales and from the surface of otoliths as described by Bilton and Jenkinson (1968). Mean diameter of fresh, ripe eggs was estimated for 21 females of each type by measuring the length of a row of 10 eggs oriented randomly (because of their asymmetry) and dividing by 10 (Craig 1985).

TABLE 1. Genetic design for sockeye and kokanee crosses. K and S denote kokanee and sockeye types, respectively. Numbers after letters are for identification of individual parents, and other numbers for identification of individual families. Only progeny of families 1–48 were reared in tanks; families 9–16 were split among two tanks each (16 tanks in total) to evaluate tank effects.

	Male			Male				Male	
Female	K1	S1	Female	K2	S2		Female	K9	S9
K1	1	2	K3	9	10		K17	65	66
S1	3	4	S3	11	12	...	S17	67	68
K2	5	6	K4	13	14		K18	69	70
S2	7	8	S4	15	16		S18	71	72

Fertilization and Rearing

Eggs were stripped from each female and split into two 4-L jars, with half the eggs to be fertilized by one male of each type. Similarly, milt from each male was split four ways and used to fertilize eggs from two females of each type (see Table 1). The four possible cross types are referred to as pure sockeye and pure kokanee (denoted PS and PK, respectively), and hybrid sockeye and hybrid kokanee (denoted HS and HK where the eggs were taken from sockeye and kokanee, respectively). The eggs were fertilized in the jars, rinsed, and transported to a hatchery in coolers chilled to about 4°C. Within 20 h of fertilization, the eggs were transferred to Heath tray baskets (one per family), disinfected, and incubated at 8.5°C in well water. The proportion of live eggs was determined at the eyed egg stage 52 d after fertilization (December 3, 1986); dead eggs were removed, and the remaining live eggs were culled randomly to 300 per family.

Alevins were transferred to 480-L tanks supplied with flowing 8.5°C well water on day 115 (February 4, 1987), after incubating for 942 degree-days. Owing to limitations on rearing capacity, only 48 of the original 72 families were transferred to separate tanks. The 48 families were selected to ensure a balanced genetic design; many families were rejected because too few eggs had survived to emergence. One family was lost shortly after transfer due to an improperly fitted drain plate. One "block" of eight families sired by one male of each type was split into two equivalent blocks to permit evaluation of tank effects. The block comprising families 9–16 (se Table 1) was selected for replication because the number of alevins was sufficient in each of these families. Random samples of five alevins from each of the 48 families (except those with fewer than about 150 fish) were preserved in 10% formalin for at least 30 d and measured for total weight (including yolk), body weight (without yolk), and standard length.

The free-swimming fry were fed Oregon Moist Pellets ad libitum dispensed by automatic feeders every 30 min during daylight on a natural photoperiod. All tanks were inspected daily and all deaths were recorded. Fork lengths and total weights of 30 fry chosen randomly from each family were measured on days 214, 352, and 460, respectively, after transfer to the 480-L tanks. The fry were anesthetized with 2-phenoxyethanol prior to measurement and were returned to their respective tanks after recovery.

Statistical Analysis

The genetic design in Table 1 is essentially two experiments, one for sockeye and one for kokanee, with two females nested

within each of the six males. However, the two experiments are not independent since all females are also crossed to males of the opposite type. This aspect of the design provides control for individual female effects but also complicates statistical analysis. Insufficient data were available to estimate parameters for the full ANOVA model, so we analyzed independent subsets of the data to test various effects.

We looked for male and female parental effects on five indices of alevin development — total weight, body weight, % body weight, KD (a condition factor index based on the ratio of total weight to length, Bams 1970), and standard length — within cross types using nested ANOVA, with females nested within males. Comparable data were not available for all families represented in Table 1 because samples of alevins were not collected if fewer than 150 had survived, and because samples of alevins from three families were lost before being measured. We analyzed individual measurements (5 fish per family), first, for 10 PS families (two females within each of five males), second, for 12 HS families (two females within each of six males), and third, for six PK families (two females within each of three males). Too few data were available to permit a similar analysis for HK families. An arcsine transformation was applied to the % body weight data to stabilize variances before analysis.

To determine whether rearing density had a significant influence on fry growth rates during our study, we tested tank density, cross type, and density × cross type interaction effects by ANCOVA using individual measurements from the eight families replicated in different tanks. We considered cross type as a treatment (ignoring family effects) and density, as a covariate. Sample variances of fry length were not always homogeneous across families, so we also tested the significance of rearing density within cross types, and of cross type, using the non-parametric Kruskal–Wallis test on the same data. Because rearing density did have significant effects on fry growth rates, especially at densities below 100 fish/tank (see Results), we restricted our attention to 37 families reared within an "acceptable" density range of 100–130 fish/tank for analyses of fry size. We did not attempt to estimate the magnitude of family effects on fry size because sample variances of fry size statistics were not homogeneous across all families, and because the number of families of each type was relatively small.

We looked for female type, male type, and female type × male type interaction effects on development and length at both the alevin and fry stages using two-way ANOVA. Comparable data (where the effects of rearing density were controlled) were available from 16 families to form four balanced, independent factorial designs (two male types × two female types). These data could not be analyzed simultaneously as a complete factorial design except by using mean values to represent each family to avoid pseudo-replication. Thus, replicated means (of 30 measurements) were available from only four independent families of each type for an analysis of family and cross type effects (analysis A). This analysis is rigorous but very conservative (i.e. has low statistical power) because it relies on mean values instead of individual measurements. To ensure the generality of our results, we repeated the analysis with a similar factorial design using data from additional families, some of which were sired by the same males, and were therefore not strictly independent (analysis B). Analysis B included mean values for alevin or fry data from each of the 37 families sampled for alevin development or reared at an acceptable density (8, 6, 12, and 11 families as alevins and 8, 8, 10, and 11 families as fry for PK, HK, HS, and PS types, respectively). In our final, most general but least rigorous analysis, we pooled all individual measurements from the 37 families by cross type, ignoring individual family effects ($n = 5 \times 37$ for alevins, and $n = 30 \times 37$ for fry), to test for cross type effects using both ANOVA and the nonparametric Kruskal–Wallis test (because variances were not always homogeneous).

Results

Differences Between Parental Types

All sockeye and kokanee parents were 4 yr old. Patterns of circuli on scales and otoliths indicated that the sockeye had all smolted in their second year and spent 2 yr at sea (age 1.2) whereas the kokanee had spent all 4 yr in fresh water (age 3.0). The sockeye parents were, on average, almost twice as long as the kokanee parents (Fig. 1). Postorbital-hypural lengths for the males of both forms were slightly greater than those of females, but this difference was only statistically significant among sockeye ($P<0.005$, t-test) in which sexual dimorphism was generally much more pronounced.

We estimated the fecundity of Shuswap sockeye and kokanee to be about 4100 and 500, respectively, based on unpublished data for Shuswap Lake sockeye (J. Woodey, Pacific Salmon Commission, 1155 Robson St., Suite 600, Vancouver, B.C., V6E 1B9, pers. comm.) and fecundity — length relationships for Shuswap Lake kokanee (C. J. Foote and C. C. Wood, unpubl. data). The average diameter of sockeye eggs collected from the Shuswap River (5.21 mm, SD = 0.25) was 7.6% larger ($P<0.001$, t-test) than that of kokanee eggs (4.84 mm, SD = 0.22), but the egg size distributions overlapped (Fig. 2). There was no significant correlation between female length and egg diameter in either sockeye or kokanee $r = -0.20$ and 0.13, respectively).

Fertilization and Survival of Fry

Both the pure and hybrid crosses produced viable progeny. However, on average, the percentage of eggs developing to the eyed stage was significantly greater ($P<0.007$, ANOVA using arcsine-transformed proportions, Table 2) for pure sockeye (PS, 86%) and hybrid sockeye (HS, sockeye female × kokanee male, 88%) than for pure kokanee (PK, 78%) or hybrid kokanee (HK, 77%). This indicates a female type effect (Fig. 3). Survival of eggs to the eyed stage was not influenced by male type ($P>0.46$), or the size of the eggs; regressions of arcsine-transformed percent survival on egg diameter were not significant within any of the four cross types ($P>0.50$, F test), but sample sizes were small.

A higher proportion of emergent fry from sockeye eggs (97.6 and 96.9% for the PS and HS crosses, respectively) survived to day 214 than those from kokanee eggs (83.0 and 90.7% for the PK and HK crosses, respectively). Again, the difference in survival was associated largely with female type ($P<0.002$, ANOVA, Table 2). Survival rates for all crosses averaged 98.8% during the remainder of the rearing period (day 214–460) with no significant differences between cross types ($P>0.36$, Table 2). Thus, mortality was generally low during this study and mortality was not higher for hybrid cross types than for pure cross types.

Size and Yolk Reserves of Alevins

Effect of cross type

On day 115, when the alevins were transferred to the large tanks, there were obvious differences between alevins from the

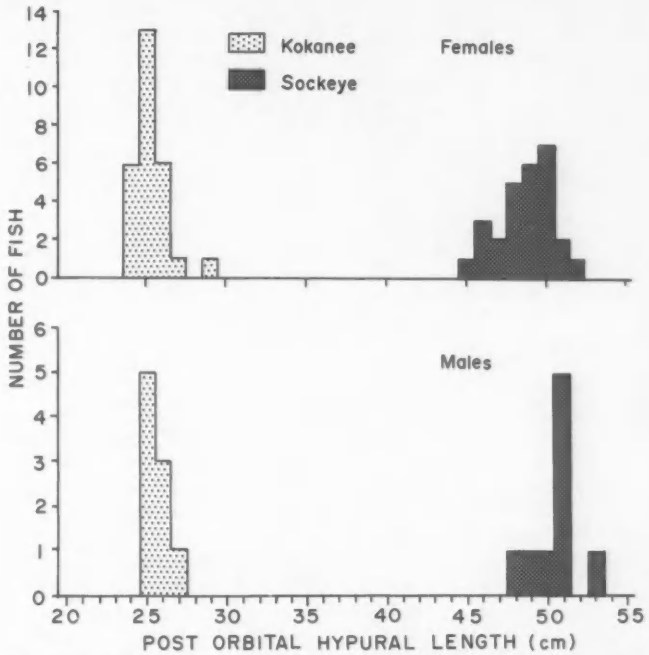

FIG. 1. Length distributions of sockeye and kokanee in the Lower Shuswap River.

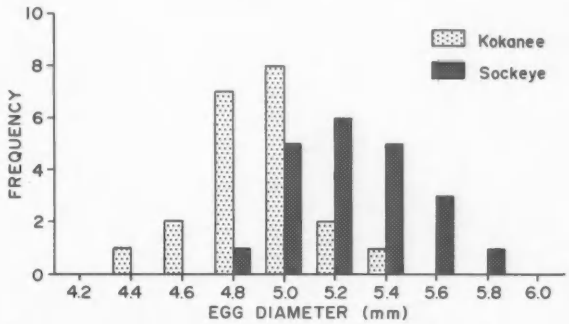

FIG. 2. Egg diameter distributions of sockeye and kokanee in the Lower Shuswap River.

four cross types (see Table 3 for statistical comparisons). Total alevin weight (including yolk) was affected by female type but not male type. Pure sockeye and HS alevins weighed more, on average, than PK and HK alevins (Fig. 4A), presumably owing to the difference in egg size between female types. Total weight did not differ significantly between PS and HS crosses, or between PK and HK crosses.

Body weight (excluding yolk) and standard length of alevins were greatest for PS, intermediate for HS, and least for PK and HK crosses but only the PS cross was significantly different. Thus, both female type and male type effects were evident. Male type effects were especially obvious with respect to the percentage of total weight accounted for by body weight (% body weight) and the KD index. Neither index was significantly different for the PS and PK crosses, indicating a similar percent conversion of yolk to body tissue despite differences in overall

TABLE 2. Statistical analyses of egg diameter and survival of alevins and fry using means and arcsine-transformed proportions, respectively, for individual families. PK and PS denote pure kokanee and pure sockeye; HK and HS, hybrid crosses (eggs from kokanee and sockeye, respectively); ft and mt denote female and male type effects. Significance levels. NS $P>0.10$; (*) $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.005$.

Variable	Order of crosses	Number of families	Effect tested		
			ft	mt	ft × mt
Egg diameter	S > K	42[a]	***	—	—
Survival					
Days 0–52[b]	PS = HS > HK = PK	72	**	NS	NS
Days 115–214	PS = HS > HK = PK	47	***	(*)	NS
Days 214–460	PS = HS = HK = PK	47	NS	NS	NS

[a]Hybrid crosses excluded.
[b]To eyed stage.

egg mass. In contrast, the two hybrid cross types lay at opposite extremes with respect to the pure cross types (Fig. 4C). Hybrid kokanee alevins had absorbed a significantly higher percentage of their yolk, and HS alevins a significantly lower percentage, than the two pure cross types. The sockeye "male type effect" becomes obvious when the confounding effect of egg size is controlled: within sockeye eggs, PS alevins were more advanced in their development (i.e. less yolk left at day 115) and longer than HS alevins; within kokanee eggs, HK alevins were more advanced but not significantly longer than PK alevins (Fig. 4B), presumably because the HK alevins had already depleted their yolk reserves beyond the point of continued growth (i.e. maximum alevin wet weight, Heming et al. 1982).

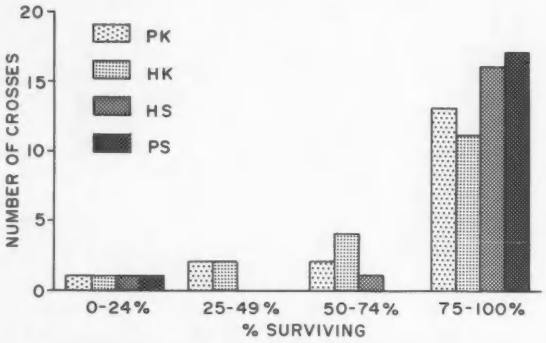

FIG. 3. Comparison of survival to the eyed-egg stage of pure sockeye (PS), pure kokanee (PK), and reciprocal hybrids (HS and HK, where S and K denote the female type).

Effect of family within cross type

Highly significant ($P<0.001$, nested ANOVA) female parental effects were detected for all five indices of alevin size and development within each of the three cross types examined (PS, HS, and PK). Male effects were not significant or only marginally significant in any of the same comparisons. We suspect that these significant differences between families arose largely from maternal effects associated with egg size or yolk. Within pure sockeye families, egg diameter was significantly correlated with total weight ($r=0.72$), body weight (0.73), and standard length ($r=0.68$), but not with % body weight ($r=-0.005$). In hybrid sockeye families the pattern of correlation was similar ($r=0.57$, 0.48, 0.45, and -0.48 for the same four indices, respectively), but no correlations were statistically significant. However, sample sizes were so small that a correlation coefficient of 0.63 was required for statistical significance. Too few data were available to examine correlations in the PK and HK families.

Growth of Fry

Effect of density

We attempted to maintain comparable densities of fish between families by culling eyed eggs to 300/family, and fry to 130/family at day 214. Even so, variations in survival of alevins among families resulted in corresponding variation in the density of fry in the rearing tanks. Families reared at low density (<100 fry/tank) clearly experienced more favourable growth than those reared near the target densities. To minimize

TABLE 3. Statistical analyses of alevin development and fry growth data using various ANOVA designs. PK and PS denote pure kokanee and pure sockeye crosses; HK and HS, hybrid crosses (eggs from kokanee and sockeye, respectively). Analysis A, $n=16$ (families); analysis B, $n=37$ (families). Significance levels: NS $P>0.10$; (*) $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.005$.

			Individual data pooled by cross type (37 families)		Factorial analyses using family means			
			Effect of cross type			Effect tested		
Life stage	Variable	Age (d)	Rank order[a]	Significance	Analysis	Female type (ft)	Male type (mt)	ft × mt
Alevin	Total weight	115	PS = HS > HK = PK	***	A	***	NS	NS
					B	***	NS	NS
	Body weight	115	PS > HS = HK = PK	***	A	***	NS	NS
					B	***	NS	NS
	% Body weight	115	HS > PK = PS > HK	***	A	***	***	(*)
					B	***	***	***
	KD index	115	HS > PK = PS > HK	***	A	***	*	NS
					B	***	(*)	(*)
	Standard length	115	PS > HS = HK = PK	***	A	***	*	NS
					B	***	(*)	(*)
Fry	Fork length	214	PS = HS = HK > PK	***	A	NS	NS	NS
					B	NS	NS	NS
		352	PS = HS = HK > PK	***	A	NS	NS	NS
					B	(*)	(*)	NS
		460	PS = HS = HK > PK	***	A	NS	NS	NS
					B	NS	NS	NS
	C.V. fork length	214	PK > HK = HS > PS	***[b]	A	NS	NS	NS
					B	*	NS	(*)
		352	PK > HK = HS > PS	***[b]	A	NS	(*)	(*)
					B	*	*	NS
		460	PK > HK = HS > PS	***[b]	A	(*)	**	NS
					B	***	**	NS

[a]An equal sign implies no statistically significant difference based on Tukey's a priori multiple comparisons. The significance level refers to the least significant of the significant differences.

[b]Coefficient of Variation (C.V.) of fork length was calculated for each of 37 families (equivalent to testing for a cross type effect using analysis B).

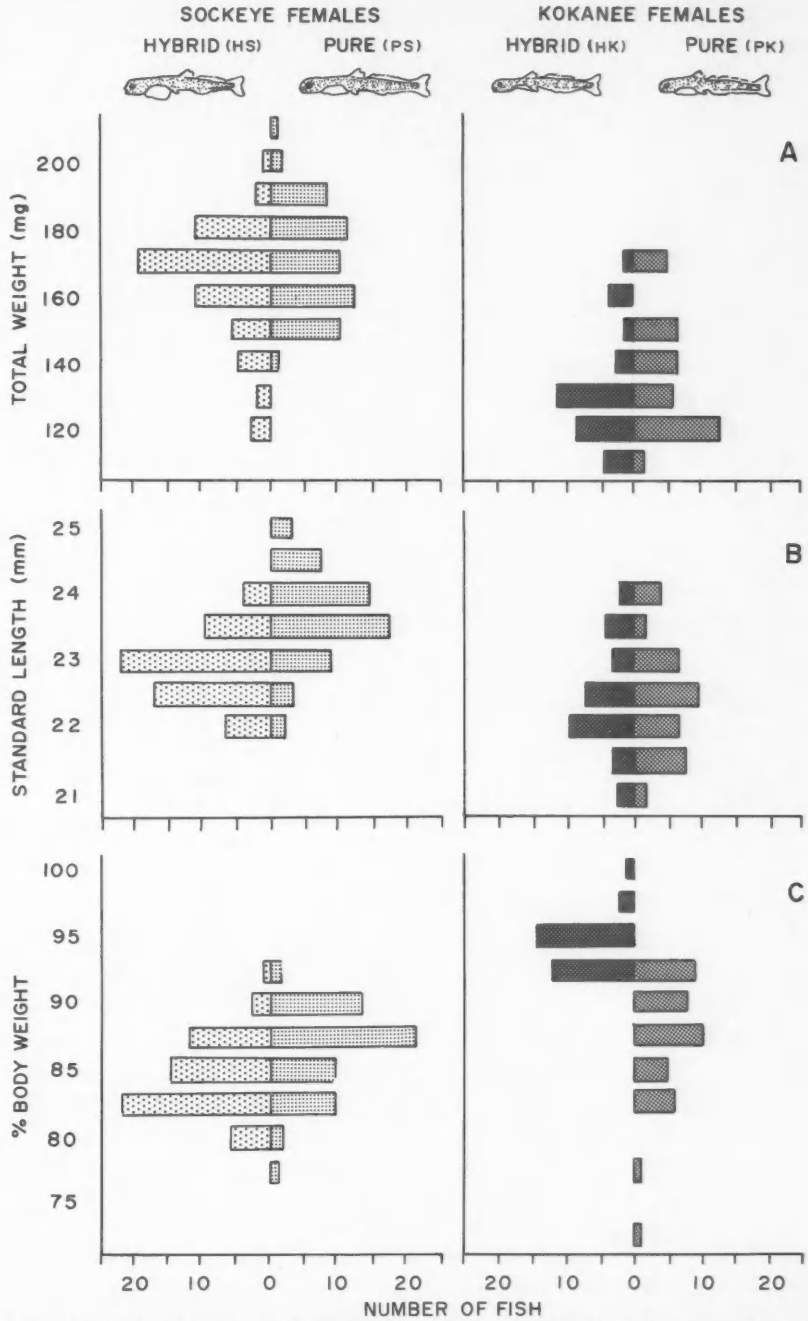

FIG. 4. Comparison of alevin development indices for pure and reciprocal hybrid crosses. (A) total alevin weight (including yolk); (B) standard length; and (C) % body weight (body weight/total weight × 100). Left column, progeny of sockeye females; right column, progeny of kokanee females.

the confounding effect density has on growth rate, subsequently, we consider only those families reared within the acceptable density range (100–130 fry/tank after day 214).

We examined the effect of density on the growth of fry reared within the acceptable density range by comparing the final length (day 460, when variation in size was maximal) attained by two groups of fry from the same family reared in different tanks at slightly different densities. These comparisons were

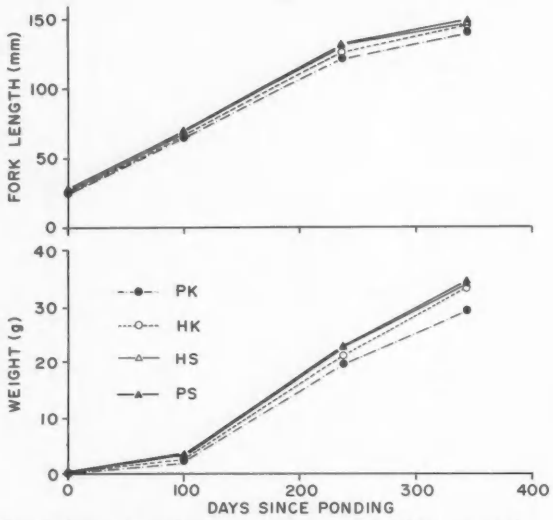

FIG. 5. Comparison of mean sizes of pure and hybrid crosses at days 115, 214, 352, and 460 after fertilization. Means were calculated by pooling measurements for 37 families by cross type (n=240, 240, 300, and 330 for PK, HK, HS, and PS crosses, respectively, at each sampling period excluding day 115). Fry were ponded at day 115.

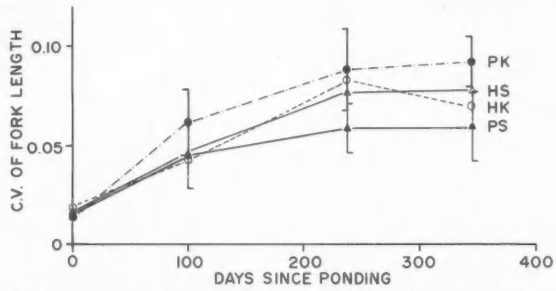

FIG. 6. Comparison of coefficients of variation (C.V.) of fork length for individual pure and hybrid families at days 115, 214, 352, and 460 after fertilization. Symbols represent overall mean C.V. across families within cross types (n=8, 8, 10, and 11 families for PK, HK, HS, and PS crosses, respectively, at each sampling period excluding day 115); error bars indicate one standard deviation around overall mean for PK and PS crosses (left and right facing bars, respectively).

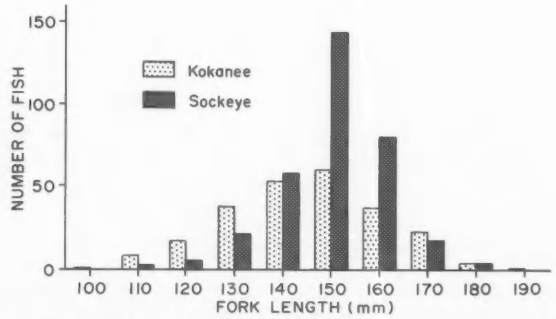

FIG. 7. Comparison of fork length distributions for pure sockeye and pure kokanee at day 460 after fertilization. Measurements were pooled across families by cross type (n=330 and 240, respectively) as in Fig. 5.

available for eight families (9–16) at densities ranging from 98 to 132 fry/tank; five of the paired comparisons differed in density by at least 13 fry/tank, and one by 26 fry/tank. However, only one of the eight replicated families reared at 113 and 130 fry/tank showed a significant difference in fork length at day 460 (P<0.05, t-test), with the fry reared at the highest density exhibiting the least growth. An analysis of covariance using the data from all eight families simultaneously, with cross type as the treatment and density as the covariate, indicates that density explained 3.3% of the total variation (P<0.001), cross type 1.8% (P<0.05), and density × cross type interactions only 0.7% (P=0.06). Because sample variances for the eight replicated families were not all homogeneous, not all assumptions in the ANCOVA were strictly satisfied. However, a nonparametric Kruskal–Wallis test gave similar results: density had a significant effect on length (P<0.001) within at least one of the four cross types (test statistic values ranged from 4.3 to 16.7 with 3 df), but cross type had a much larger effect (test statistic = 88.7 with 3 df).

We conclude that even small differences in rearing density can influence growth rate. Even so, the effects of differences in rearing density within the acceptable range are small enough that they should not obscure genetic effects on length, given that similar variation in density occurred among all cross types.

Effect of cross type

Growth rates between days 214 and 460 were generally similar among all cross types (Fig. 5). Pure sockeye families tended to be largest, HS and HK families intermediate, and PK families smallest at each sampling period (P<0.001 for all ANOVA and Kruskal–Wallis tests except one with P<0.02, using data pooled across families by cross type, Table 3). However, the differences between cross types were so small that large sample sizes were required to detect statistically significant differences. Thus, the factorial analyses using only the mean values for each family (analyses A, n=4/cell and B, n=8 to 11/cell) failed to detect significant male-type or female-type effects. The differences in size among crosses tended to increase over time (Fig. 5 and Table 3), and in particular, PK families grew more slowly than other families during the interval from day 352 to day 460.

The variation in fork length within PK families tended to be greater than that within PS families. Hybrid families displayed intermediate variation in fork length with no obvious differences between HS and HK crosses. This pattern was evident not only across families (i.e. for the standard deviations of measurements pooled by cross type), but also within families (for the arcsine-transformed coefficients of variation, C.V., of mean length of individual families) (P<0.001, ANOVA and Kruskal–Wallis test, Fig. 6). Both female type and male type effects were significant in factorial analyses A and B (Table 3). As with size, differences in the variation in size among cross types increased over time.

The fork length distributions for all PS and PK families reared within the acceptable density range and pooled by cross type indicate that the fastest growing individuals of both types were similar in size at all sampling periods (Fig. 7). However, the slowest growing individuals were smaller in PK crosses than in PS crosses such that the PK length distributions were broader. The length distribution for PK crosses became progressively more skewed towards the small sizes over time, relative to the

corresponding distribution for PS families. The differences in shape and relative position of these length distributions account for the differences in variance and mean of fork length among cross types noted earlier.

Discussion

Sympatric sockeye and kokanee from the Shuswap River differ in a number of respects that could have important demographic implications. The most obvious differences between the types are morphological: sockeye are about twice as long as kokanee, their fecundity is about eight times greater and their egg diameters are slightly larger. A great difference in fecundity was expected given the strong positive relationship between fecundity and length in sockeye (Foerster 1968; Bilton 1971; Manzer and Miki 1986) and other salmonids (Bagenal 1967; Healey and Heard 1984). A strong positive relationship also exists between egg size and female length in sockeye and other Pacific salmon (Foerster and Pritchard 1941; Bilton and Jenkinson 1966; van den Berghe and Gross 1989). Thus, the relatively slight difference in egg size between sockeye and kokanee was unexpected, and indicated that egg size has been conserved relative to body size in the smaller kokanee (see also Murray et al. 1990). However, we do not know whether the relationship between egg size and body size is different for the two types, or whether both types share the same non-linear relationship.

Comparison of Egg to Fry Survival

The difference in egg size between sockeye and kokanee, although small, probably accounted for many of the female type effects observed in this study, including survival to the eyed egg stage, and to day 214. Although the lower survival of kokanee eggs to the eyed stage could indicate a greater sensitivity to handling or failure to select ripe eggs (as suggested by McCart 1970), their lower survival to day 214 probably indicates a real difference in viability between sockeye and kokanee fry at ponding (day 115). Virtually all mortality during this latter period involved "pin-head fry" (i.e. emaciated fry that do not feed after yolk absorption). The prevalence of pin-head fry was inversely related to egg size and alevin size in Arctic charr (*Salvelinus alpinus*) (Wallace and Aasjord 1984); also, brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*)[2] alevins from large eggs survived better than those from small eggs (Bagenal 1969; Kincaid et al. 1977; Pitman 1979). Thus, the difference in survival of kokanee and sockeye eggs and alevins was probably also related to egg size. However, we could not detect a significant effect of egg size on survival within cross types given our small sample sizes and the limited variation in size within types.

Comparison of Alevin Development

Egg size can account for much variation in the size of alevins at emergence in salmonids (Bilton 1971; Fowler 1972; Pitman 1979; Robison and Luempert 1984; Wallace and Aasjord 1984; Beacham and Murray 1985; Beacham et al. 1985; Rombough 1985; McKay et al. 1986) and in the development rate of alevins (Smith 1958; Bagenal 1969; Rombough 1985; Heming et al. 1982). In this study, strong maternal effects were evident; alevins from sockeye eggs were longer and heavier than those from kokanee eggs. However, we saw no evidence that the progeny of pure sockeye and pure kokanee crosses developed at different rates, as might be expected from the differences in their respective egg sizes (see Rombough 1985). At ponding, pure sockeye and pure kokanee alevins were similar in both the % body weight and KD indices of development.

Whereas pure kokanee and pure sockeye alevins developed at the same rate, as indicated by the % body weight and KD indices, the reciprocal hybrid type alevins differed both from pure type alevins and from one another. The progeny of kokanee females and sockeye males (HK) had the least proportion of yolk left at ponding and the lowest KD values, whereas the opposite was true for the progeny of sockeye females and kokanee males (HS). These results indicated strong genetic and probable maternal type effects on development. Within female types (where differences in egg size were controlled), yolk was absorbed faster in families sired by sockeye than those sired by kokanee, indicating genetic differences between male types. Similar male type genetic effects have been observed in crosses between Pacific salmon species (Withler and Morley 1970). Within male type (where differences in egg size were not controlled), the progeny of kokanee females developed faster than those of sockeye females. While these female type differences are in the opposite direction to that indicated by the male type genetic effects, they are in the direction expected based on the differences in egg size between sockeye and kokanee. Hence, the developmental phenotypes observed in the different crosses appear to result from the combination of male genetic effects and maternal (egg size) effects. Any female type genetic effects probably would have been obscured by the differences in egg size.

That pure sockeye and pure kokanee crosses developed at the same rate despite differences in egg size indicates that the two forms are genetically different in a way that compensates for the effects of egg size on development, thereby producing the same developmental phenotype. Assuming that differences in egg size between sockeye and kokanee have evolved in response to differences in their life history, it appears that development rates have been fine tuned genetically to ensure that fry of the two forms develop and emerge at the same time. Brannon (1987) arrived at the same conclusion after finding that egg size and time to yolk absorption were positively correlated ($r = 0.88$) in comparisons of families from different sockeye populations in the Fraser River. Brannon (1987) also reported that in Cultus Lake, eggs collected from the earliest spawning sockeye required more time to complete yolk absorption than eggs of similar weight collected from the latest part of the run and incubated under identical conditions; eggs taken in the middle of the run required an intermediate time for development. Similar results have been reported for chum salmon (*Oncorhynchus keta*) (Tallman 1988) and Atlantic salmon (*Salmo salar*) (Heggberget 1988). Collectively, these results provide strong evidence that emergence timing in salmonids is under stabilizing selection. However, for emergence timing to be stabilized within sympatric sockeye and kokanee populations in the Lower Shuswap River there must also be disruptive selection on the developmental genotype to compensate for the effect of differences in their respective egg sizes.

Comparison of Fry Growth

We conclude that size at age was influenced by cross type, but that the effect was small. In fact, it is perhaps more sur-

[2]Formerly *Salmo gairdneri*.

prising that the average sizes of fry in all cross types were so similar considering the initial differences in the size of alevins. Other investigators (Bilton 1971; Reagan and Conley 1977; Thorpe et al. 1984; McKay et al. 1986) have also reported that early differences in size associated with egg mass may not persist for long when food is abundant.

Cross type did, however, influence the variation in growth within families, with pure kokanee families showing the greatest variation in length, and pure sockeye families the least, at all sampling periods. This variability is influenced by both male type and female type, with the type effects increasing over time. The conclusion that differences in variation within families result from genetic differentiation between the types is supported by the fact that variation within hybrid families was intermediate to that in pure families as predicted by a simple genetic model. Moreover, other explanations appear implausible. First, the initial variation in egg and alevin sizes was similar among cross types, indicating that the difference in variation in fry size developed over time. Second, the variation in fry size could not have been an artifact of rearing density because density varied randomly among cross types within very narrow (controlled) limits. Finally, the variation in fry size probably was not caused by differences in aggressive activity among cross types because we observed no overt aggression in any of the rearing tanks. *Oncorhynchus nerka* is considered to be a schooling, non-aggressive, species (Hoar 1976).

We suggest that reduced variability in growth rates within sockeye compared to that within kokanee results from stronger directional selection for large size in sockeye. Fast growth (i.e. large size) enhances the survival of sockeye smolts during their seaward migrations (Ricker 1962; Burgner 1964; Foerster 1968; Koenings and Burkett 1987a) and probably during their return migrations also (Ricker 1972, 1987). For example, in Atlantic salmon, size at maturity is positively correlated with the difficulty of migration to the spawning stream (Schaefer and Elson 1975; Thorpe and Mitchell 1981). In addition, Taylor and McPhail (1985b) demonstrated that coho salmon (*Oncorhynchus kisutch*) fry from the interior of British Columbia were stronger swimmers than fry of similar size from coastal streams. They attributed these differences to stronger selection for swimming performance in populations undertaking longer migrations. Given that kokanee do not undertake long migrations, selection for fast growth and swimming performance may be relaxed (see also Taylor and Foote 1991).

Mortality of Hybrid Progeny in Nature

Our results clearly indicate that Shuswap River sockeye and kokanee differ genetically in ways that affect rates of development and growth. These findings corroborate Foote et al.'s (1989b) inferences based on differences in allozyme allele frequencies. Hence, we conclude that the observed differences in allele frequency were not simply an artifact of natural selection on allozymes; rather these data provide reliable evidence that in the three lakes examined, sockeye and kokanee are genetically-distinct sympatric populations.

We found no direct evidence in this study to confirm our hypothesis that genetic differences between sympatric sockeye and kokanee are maintained because the progeny of hybrid crosses between sympatric sockeye and kokanee are less viable than those of pure cross types. Survival under hatchery conditions was generally high for all cross types. Perhaps this should not be surprising considering that even hybrids between different species of salmonids can thrive in hatcheries (Withler and Morley 1970; Ayles 1974; Goddard and Tait 1976; Ord et al. 1976). Similarly, Nordeng (1983) noted no differences in survival of progeny of crosses between anadromous and non-anadromous Arctic charr.

Even so, observed genetic differences in the rate of early development and growth among cross types would likely influence their survival under more natural conditions where food supply can limit survival, and where alevins and fry would be vulnerable to predation and disease (Ord et al. 1976). In nature, mortality during the first year from emergence is typically >90% (e.g. Foerster 1968) compared with mortalities of 4–20% observed in this study. Timing of emergence is thought to be critical for survival in many fishes, including salmon, because the risk of starvation can be minimized, and the opportunities for growth maximized, by synchronizing emergence with forage production (Foerster 1968; LeBrasseur et al. 1969; Koenings and Burkett 1987b). Synchronization of emergence within a population also reduces the risk of predation for individual fry by swamping predators such that overall mortality is depensatory (Neave 1953; Ward and Larkin 1964; Ginetz and Larkin 1976; Ruggerone and Rogers 1984; Mace 1983; Wood 1987).

Interbreeding between sockeye and kokanee in the Lower Shuswap River (and likely in all Shuswap Lake tributaries where the forms spawn sympatrically) would result in hybrid alevins with "mismatched" egg size and development rates. That is, the male type effect on rate of yolk absorption would tend to exaggerate rather than compensate for the female type effect of initial egg size. For example, the vast majority of interbreeding between the forms in the wild must occur when kokanee males sneak in on the spawnings of sockeye pairs (McCart 1970; Foote and Larkin 1988). Hybrid sockeye resulting from such sneaking would have proportionately much larger yolk reserves than both pure sockeye and pure kokanee alevins after a normal period of incubation because embryonic development was delayed. We do not know whether hybrid sockeye and pure sockeye resulting from the same crosses would emerge at the same time, or whether hybrid sockeye would delay emergence until they had developed further, but in either case, hybrid fry would appear to be at a disadvantage compared with pure fry. On the one hand, hybrid fry emerging at the same time would be encumbered by larger yolk sacs and smaller size (body weight and fork length), and hence, would be more vulnerable to predation (Bams 1967; Fresh and Schroder 1987). Similarly, there is much evidence of size-selective mortality in salmon (Healey 1982; Taylor and McPhail 1985a; West and Larkin 1987). Because mortality from predation can be extreme shortly after emergence (e.g. 75%, Foerster 1968), selective predation on small fry encumbered by large yolks may virtually eliminate gene flow between the forms. On the other hand, if hybrid sockeye delay emergence, they may be disadvantaged in competition for food with pure sockeye and kokanee which had emerged earlier and presumably had grown larger. In addition, hybrid sockeye may be subject to increased predation pressure if they emerge at a time when the majority of sockeye and kokanee have left, since the swamping of predators will be decreased.

Thus, it seems likely that hybrid fry would survive less well than pure fry in nature and that selective mortality on hybrid fry would reduce gene flow between the forms in the Shuswap River. In addition, preliminary comparisons of the hybrid and pure forms with respect to seawater adaptability (Foote et al.

1989a), swimming performance (Taylor and Foote 1991) and age at maturation (C. Wood and C. Foote, unpubl. data) have revealed other genetic differences on which selection might act to reduce or eliminate gene flow between the sympatric types in nature.

This study provides further evidence for the genetic differentiation of sympatric forms of the same species. We believe the differentiation of sockeye and kokanee is promoted by assortative mating by size (Hanson and Smith 1967; Foote 1988, Foote and Larkin 1988), and by selection against hybrid progeny owing to great selective differences between the marine and lacustrine environments (Ricker 1940), and the special adaptations required for anadromy. Mate selection behaviour of sockeye and kokanee in the Shuswap River has not yet been studied, but premating isolating mechanisms between the types (in addition to those based on size alone) have been demonstrated in two other lake systems where the forms spawn sympatrically (Foote 1987; Foote and Larkin 1988). Further study is needed to determine what degree of selective advantage of the pure forms over hybrid forms is required to initiate and maintain the process of genetic differentiation observed in sympatric sockeye and kokanee populations.

Acknowledgements

We are especially grateful to D. Rutherford, D. Brouwer, B. Humphreys, G. Johnston, G. Ball, and Z. Rogak for technical assistance throughout this study, and to C. Hand and K. Rutherford for assistance during broodstock collection. Two anonymous referees provided useful comments on the manuscript.

References

AYLES, G. B. 1974. Relative importance of additive genetic and maternal sources of variation in early survival of young splake hybrids (*Salvelinus fontinalis* × *S. namaycush*). J. Fish. Res. Board Can. 31: 1499–1502.

BAGENAL, T. B. 1967. A short review of fish fecundity, p. 89–111. *In* S.D. Gerking [ed.] The biological basis of freshwater fish production. Blackwell, Oxford, UK.

1969. Relationship between egg size and fry survival in brown trout *Salmo trutta* L. J. Fish Biol. 1: 349–353.

BAMS, R. A. 1967. Differences in performance of naturally and artificially propagated sockeye salmon migrant fry, as measured with swimming and predation tests. J. Fish. Res. Board Can. 24: 1117–1153.

1970. Evaluation of a revised hatchery method tested on pink and chum fry. J. Fish. Res. Board Can. 27: 1429–1452.

BEACHAM, T. D., AND C. B. MURRAY. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia. Can. J. Fish. Aquat. Sci. 42: 1755–1765.

BEACHAM, T. D., F. C. WITHLER, AND R. B. MORLEY. 1985. Effect of egg size on incubation time and alevin and fry size in chum salmon (*Oncorhynchus keta*) and coho salmon (*O. kisutch*). Can. J. Zool. 63: 847–850.

BILTON, H. T. 1971. A hypothesis of alternation of age at return in successive generations of Skeena River sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 28: 513–516.

BILTON, H. T., AND D. W. JENKINSON. 1966. Relationship between egg size and fish size in sockeye salmon (*Oncorhynchus nerka*). Fish. Res. Board Can. MS Rep. 1096: 8 p.

1968. Comparison of otolith and scale methods for aging sockeye (*Oncorhynchus nerka*) and chum (*O. keta*) salmon. J. Fish. Res. Board Can. 25: 1067–1069.

BRANNON, E. L. 1987. Mechanisms stabilizing salmonid fry emergence timing, p. 120–124. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

BURGNER, R. L. 1964. Factors affecting production of sockeye salmon (*Oncorhynchus nerka*) in lakes of Southwestern Alaska. Verh. Int. Ver. Limnol. 15: 504–513.

CHEBANOV, N. A., N. V. VARNAVSKAYA, AND V. R. VARNAVSKIY. 1983. Effectiveness of spawning male sockeye salmon, *Oncorhynchus nerka*, of differing hierarchical rank by means of genetic-biochemical markers. J. Ichthyol. 23: 51–55.

CRAIG, P. C. 1985. Identification of sockeye salmon (*Oncorhynchus nerka*) stocks in the Stikine River based on egg size measurements. Can. J. Fish. Aquat. Sci. 42: 1696–1701.

FOERSTER, R. E. 1968. The sockeye salmon. Bull. Fish. Res. Board Can. 162: 422 p.

FOERSTER, R. E., AND A. L. PRITCHARD. 1941. Observations on the relation of egg content to total length and weight in the sockeye salmon (*Oncorhynchus nerka*) and the pink salmon (*O. gorbuscha*). Trans. R. Soc. Can. Sec. V 35: 51–60.

FOOTE, C. J. 1987. An experimental examination of behavioural isolation between sockeye salmon and kokanee, the anadromous and nonanadromous forms of *Oncorhynchus nerka*. Ph.D. thesis, University of British Columbia, Vancouver, B.C.

1988. Male mate choice dependent on male size in salmon. Behaviour 106: 63–80.

FOOTE, C. J., AND P. A. LARKIN. 1988. The role of male choice in the assortative mating of sockeye salmon and kokanee, the anadromous and nonanadromous forms of *Oncorhynchus nerka*. Behaviour 106: 43–62.

FOOTE, C. J., C. C. WOOD, C. CLARKE, AND J. BLACKBOURN. 1989a. The saltwater adaptability of sympatric anadromous and nonanadromous sockeye salmon and their 'hybrids'. Aquaculture 82: 377.

FOOTE, C. J., C. C. WOOD, AND R. E. WITHLER. 1989b. Biochemical genetic comparison of sockeye salmon and kokanee, the anadromous and nonanadromous forms of *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. 46: 149–158.

FOWLER, L. G. 1972. Growth and mortality of fingerling chinook salmon as affected by egg size. Prog. Fish-Cult. 34: 66–69.

FRESH, K. L., AND S. L. SCHRODER. 1987. Influence of the abundance, size, and yolk reserves of juvenile chum salmon (*Oncorhynchus keta*) on predation by freshwater fishes in a small coastal stream. Can. J. Fish. Aquat. Sci. 44: 236–243.

FUTUYMA, D. J., AND G. C. MAYER. 1980. Non-allopatric speciation in animals. Syst. Zool. 29: 254–271.

GINETZ, R. M., AND P. A. LARKIN. 1976. Factors affecting rainbow trout (*Salmo gairdneri*) predation on migrant fry of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 33: 19–24.

GODDARD, C. J., AND J. S. TAIT. 1976. Preferred temperatures of F_3 to F_5 hybrids of *Salvelinus fontinalis* × *S. namaycush*. J. Fish. Res. Board Can. 33: 197–202.

HANSON, A. J., AND H. D. SMITH. 1967. Mate selection in a population of sockeye salmon (*Oncorhynchus nerka*) of mixed age groups. J. Fish. Res. Board Can. 24: 1955–1977.

HEALEY, M. C. 1982. Timing and relative sensitivity of size selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can. J. Fish. Aquat. Sci. 39: 952–957.

HEALEY, M. C., AND W. R. HEARD. 1984. Inter- and intra-population variation in the fecundity of chinook salmon (*Oncorhynchus tshawytscha*) and its relevance to life history theory. Can. J. Fish. Aquat. Sci. 41: 476–483.

HEGGBERGET, T. G. 1988. Timing of spawning in Norwegian Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 45: 845–849.

HEMING, T. A., J. E. MCINERNEY, AND D. F. ALDERDICE. 1982. Effect of temperature on initial feeding in alevins of chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 39: 1554–1562.

HOAR, W. S. 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish. Res. Board Can. 33: 1234–1252.

HUTCHINGS, J. A., AND R. A. MYERS. 1988. Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. Oecologia 75: 169–174.

KINCAID, H. L., W. R. BRIDGES, AND B. VON LIMBACH. 1977. Three generations of selection for growth rate in fall-spawning rainbow trout. Trans. Am. Fish. Soc. 106: 621–628.

KOENINGS, J. P., AND R. D. BURKETT. 1987a. Population characteristics of sockeye salmon (*Oncorhynchus nerka*) smolts relative to temperature regimes, euphotic volume, fry density, and forage base within Alaskan lakes, p. 216–234. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

1987b. An aquatic Rubic's cube: restoration of the Karluk Lake sockeye salmon (*Oncorhynchus nerka*), p. 419–434. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

LeBrasseur, R. J., W. E. Barraclough, V. O. Kennedy, and T. R. Parsons. 1969. Production studies in the Strait of Georgia. Part III. Observations on the food of larval and juvenile fish in the Fraser River Plume, February to May, 1967. J. Exp. Biol. Ecol 3: 51–61.

Mace, P. M. 1983. Predator-prey functional responses and predation by staghorn sculpins (*Leptocottus armatus*) on chum salmon (*Oncorhynchus keta*). Ph.D. thesis. University of British Columbia, Vancouver, B.C.

Maekawa, K., and H. Onozato. 1986. Reproductive tactics and fertilization success of mature male Miyabe charr, *Salvelinus malma miyabei*. Envir. Biol. Fishes 15: 119–129.

Manzer, J. I., and I. Miki. 1986. Fecundity and egg retention of some sockeye salmon (*Oncorhynchus nerka*) in British Columbia. Can. J. Fish. Aquat. Sci. 43: 1643–1655.

Maynard Smith, J. 1966. Sympatric speciation. Am. Nat. 100: 637–650.

Mayr, E. 1970. Populations, species and evolution. Harvard University Press, Cambridge, MA.

McCart, P. 1970. A polymorphic population of *Oncorhynchus nerka* in Babine Lake, British Columbia. Ph.D. thesis. University of British Columbia, Vancouver, B.C.

McKay, L. R., P. E. Ihssen, and G. W. Friars. 1986. Genetic parameters of growth in rainbow trout, *Salmo gairdneri*, prior to maturation. Can. J. Genet. Cytol. 28: 306–312.

Murray, C. B., J. D. McPhail, and M. L. Rosenau. 1990. Reproductive and developmental biology of kokanee from Upper Arrow Lake, British Columbia. Trans. Am. Fish. Soc. 118: 503–509.

Neave, F. 1953. Principles affecting the size of pink and chum salmon populations in British Columbia. J. Fish. Res. Board Can. 9: 450–491.

Nelson, J. A. 1968. Variation in gillraker number in North American kokanee, *Oncorhynchus nerka*. J. Fish. Res. Board Can. 25: 415–420.

Nordeng, H. 1983. Solution to the "char problem" based on Arctic char (*Salvelinus alpinus*) in Norway. Can. J. Fish. Aquat. Sci. 40: 1372–1387.

Ord, W. M., M. LeBerre, and P. de Kinkelin. 1976. Viral hemorrhagic scepticemia: comparative susceptibility of rainbow trout (*Salmo gairdneri*) and hybrids (*S. gairdneri* × *Oncorhynchus kisutch*) to experimental infection. J. Fish. Res. Board Can. 33: 1205–1208.

Pimm, S. L. 1979. Sympatric speciation: a simulation model. Biol. J. Linn. Soc. 11: 131–139.

Pitman, R. W. 1979. Effect of female age and egg size on growth and mortality in rainbow trout. Prog. Fish-Cult. 41: 202–204.

Reagan, R. E., Jr., and C. M. Conley. 1977. Effect of egg diameter on growth of channel catfish. Prog. Fish-Cult. 39: 133–134.

Rice, W. R. 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. Evolution 38: 1251–1260.

Ricker, W. E. 1940. On the origin of kokanee, a fresh-water type of sockeye salmon. Trans. R. Soc. Can. 34: 121–135.

1962. Comparison of ocean growth and mortality of sockeye salmon during their last two years. J. Fish. Res. Board Can. 19: 531–560.

1972. Heredity and environmental factors affecting certain salmonid populations, p. 19–160. *In* R. C. Simmon and P. A. Larkin [ed.] The stock concept in Pacific salmon. University of British Columbia Press, Vancouver, B.C.

1987. Effects of the fishery and of obstacles to migration on the abundance of Fraser River sockeye salmon (*Oncorhynchus nerka*). Can. Tech. Rep. Fish. Aquat. Sci. 1522: 75 p.

Robison, O. W., and L. G. Luempert, III. 1984. Genetic variation in weight and survival of brook trout (*Salvelinus fontinalis*). Aquaculture 38: 155–170.

Rombough, P. J. 1985. Initial egg weight, time to maximum alevin wet weight, and optimal ponding times for chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 42: 287–291.

Rosenzweig, M. L. 1978. Competitive speciation. Biol. J. Linn. Soc. 10: 275–289.

Ruggerone, G. T., and D. E. Rogers. 1984. Arctic char predation on sockeye salmon smolts at Little Togiak River, Alaska. Fish. Bull. 82: 401–410.

Schaefer, W. M., and P. F. Elson. 1975. The adaptive significance of variations in life-history among local populations of Atlantic salmon in North America. Ecology 56: 577–590.

Schroder, S. L. 1982. The influence of intrasexual competition on the distribution of chum salmon in an experimental stream, p. 275–285. *In* E. L. Brannon and E. O. Salo. [ed.] Proc. of the salmon and trout migratory symposium. University of Washington Press, Seattle, WA.

Smith, S. 1958. Yolk utilization in fishes, p. 33–53. *In* D. Rudnick [ed.] Embryonic nutrition. University of Chicago Press, Chicago, IL.

Tallman, R. F. 1988. Seasonal isolation and adaptation among chum salmon, *Oncorhynchus keta* (Walbaum), populations, Ph.D. thesis, University of British Columbia, Vancouver, B.C.

Taylor, E. B., and C. J. Foote. 1991. Critical swimming velocities of juvenile sockeye salmon and kokanee, the anadromous and non-anadromous forms of *Oncorhynchus nerka* (Walbaum). J. Fish Biol. (In press)

Taylor, E. B., and J. D. McPhail. 1985a. Burst swimming and size-related predation of newly emerged coho salmon (*Oncorhynchus kisutch*). Trans. Am. Fish. Soc. 114: 546–551.

1985b. Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42: 2029–2033.

Templeton, A. R. 1981. Mechanisms of speciation — a population genetic approach. Ann. Rev. Ecol. Syst. 12: 23–48.

Thorpe, J. E., M. S. Miles, and D. S. Keay. 1984. Development rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. Aquaculture 43: 289–306.

Thorpe, J. E., and K. A. Mitchell. 1981. Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness, and current management. Can. J. Fish. Aquat. Sci. 38: 1576–1590.

van den Berghe, E. P., and M. R. Gross. 1989. Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution 43: 125–140.

Wallace, J. C., and D. Aasjord. 1984. An investigation of the consequences of egg size for the culture of Arctic charr, *Salvelinus alpinus* (L.). J. Fish. Biol. 24: 427–435.

Ward, F. J., and P. A. Larkin. 1964. Cyclic dominance in Adams River sockeye salmon. Int. Pac. Salmon Fish. Comm. Progr. Rep. 11: 116 p.

West, C. J., and P. A. Larkin. 1987. Evidence for size-selective mortality of juvenile sockeye salmon (*Oncorhynchus nerka*) in Babine Lake, British Columbia. Can. J. Fish. Aquat. Sci. 44: 712–721.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Ann. Rev. Ecol. Syst. 20: 249–278.

Withler, F. C., and R. B. Morley. 1970. Sex-related parental influences on early development of Pacific salmon. J. Fish. Res. Board Can. 27: 2197–2214.

Wood, C. C. 1987. Predation of juvenile Pacific salmon by the common merganser (*Mergus merganser*) on eastern Vancouver Island. I: Predation during the seaward migration. Can. J. Fish. Aquat. Sci. 44: 941–949.

Relative Importance of Seasonal, Short-Term pH Disturbances During Discharge Variation on a Stream Ecosystem

Ronald J. Hall

Dorset Research Centre, Limnology Section, Ontario Ministry of the Environment, Dorset, Ontario P0A 1E0 Canada

Hall, R. J. 1990. Relative importance of seasonal, short-term pH disturbances during discharge variation on a stream ecosystem. Can. J. Fish. Aquat. Sci. 47: 2261–2274.

Effects of short-term, experimental pH reduction on water chemistry and structure of benthic invertebrate communities were investigated in a south-central Ontario stream during spring and fall. With increased acidity, inorganic monomeric Al and Ca concentrations were significantly increased in stream water during spring and fall, whereas Fe, Mn, and Zn concentrations did not change. Total Al and DOC concentrations in stream water showed no constant pattern. Benthic density and generic richness during spring decreased in mineral, but not organic sediments. Total drift density did not change, but mayflies increased in the drift. In contrast, in the fall, benthic density and generic richness fluctuations were not correlated with pH disturbance, whereas drift density increased significantly during elevated acidity. Diversity and evenness of benthic invertebrates did not change above or below pH disturbance in either season. Non-metric multidimensional scaling analysis showed that discharge was the primary correlate of spring drift, whereas pH played a secondary role. In autumn, analyses demonstrated that Ca and DOC were predominant factors associated with increased drift but were related to hydrology. This study highlights the interrelationship between life history strategies of benthic invertebrates and the frequency and intensity of physical (discharge fluctuations) and chemical (pH depressions) disturbances.

Les effets de la réduction expérimentale du pH à court terme sur la chimie de l'eau et sur la structure des communautés d'invertébrés benthiques ont fait l'objet de recherches dans un cours d'eau du centre-sud de l'Ontario, au printemps et à l'automne. Avec l'accroissement de l'acidité, les concentrations de monomères inorganiques de Al et de Ca étaient considérablement plus importantes dans le cours d'eau au printemps et à l'automne, alors que les concentrations de Fe, Mn et Zn n'ont pas changé. Les COD et les concentrations d'Al total dans l'eau du cours d'eau n'ont pas affiché de profil constant. La densité benthique et l'abondance des espèces au printemps ont diminué dans les sédiments minéraux mais non dans les sédiments organiques. La densité totale des dépôts n'a pas changé, mais le nombre d'éphéméroptères a augmenté dans les dépôts. Par contre, les fluctuations relatives à la densité benthique et à l'abondance des espèces n'étaient pas corrélées avec la perturbation du pH à l'automne, alors que la densité des dépôts s'est accrue considérablement lorsque l'acidité était élevée. La diversité et l'uniformité des invertébrés benthiques n'ont pas changé au-dessus et au-dessous du niveau de perturbation du pH au cours des deux saisons. L'analyse multidimensionnelle non métrique révélé que le débit était l'élément de corrélation primaire des dépôts printaniers et que le pH pour sa part avait joué un rôle secondaire. À l'automne, des analyses ont montré que le Ca et les COD étaient des facteurs prédominants associés à l'augmentation des dépôts, mais qu'ils étaient reliés à l'hydrologie. Cette étude met en lumière les rapports réciproques qui existent entre les stratégies basées sur l'historique de la vie des invertébrés benthiques, d'une part, et la fréquence et l'intensité des perturbations physiques (fluctuations du débit) et chimiques (diminutions du pH), d'autre part.

Received November 29, 1988
Accepted May 10, 1990
(J9961)

Reçu le 29 novembre 1988
Accepté le 10 mai 1990

Short-term pH reductions from pH 6.0 to 4.0 have been reported in poorly buffered upland streams in Scandinavia (Gjessing et al. 1976) and eastern North America (Jeffries et al. 1979; Keller 1983; Sharpe et al. 1984). These disturbances result from release of protons to streams during snowmelt, acidic rain storms (Scheider et al. 1979; Silabee and Larson 1982; Corbett and Lynch 1982), and precipitation events involving reoxidation of reduced sulfur compounds in the soil following long dry periods in summer and fall (P. J. Dillon, Ontario Ministry of the Environment, Dorset, Ont., pers. comm.). Most of the above stream investigations, however, included chemical changes only; concomitant responses of benthic communities were rarely quantified.

Many surveys have documented reduced distribution and abundance of benthic invertebrates at low pH (MacKay and Kersey 1985; Peterson et al. 1985; Simpson et al. 1985). These investigators, however, could not determine whether the observed variation in community structure was the direct result of elevated H^+ concentration or the indirect effect of mobilized metals or some other factor. Further, many previous surveys have compared faunas of acidic and non-acidic streams with no consideration of temporal variability in pH. Several investigations in which natural streams (Hall et al. 1980b) or artificial channels (Burton et al. 1982; Allard and Moreau 1987) had been experimentally acidified were conducted for a relatively long time period (3–8 mo), whereas others were shorter in duration (24 h or less) (Herricks and Cairns 1974; Hall et al. 1987; Ormerod et al. 1987; Weatherley et al. 1988; Hopkins et al. 1989), but no quantitative experimental studies involving pH decreases have been made at different seasons in the same

stream. Seasonal investigation, particularly fall vs. spring, is important because many stream invertebrates populate different habitats during various life stages (Hall et al. 1980a; Giberson and Hall 1988) and because different life stages may not respond similarly to pH disturbance and associated chemical changes (Hall et al. 1988; Rowe et al. 1989).

This study had three primary objectives: (1) to evaluate the effects of episodic acid inputs on metal and DOC concentrations in stream water, (2) to quantify the effects of short-term pH disturbances on the structure of stream benthic communities, and (3) to examine seasonal effects on benthos produced by elevated acidity during spring and fall. I planned to test the hypothesis that fall invertebrate communities, relative to those in spring, would be more severely affected because organisms are smaller and more vulnerable to pH disturbances.

Study Site

Plastic Lake is a small (32.1 ha) headwater lake located on the Precambrian Shield in Haliburton County, south-central Ontario (45°11′ latitude, 78°50′ longitude) (Fig. 1). The catchment surrounding the lake has exposed rock outcrops of igneous (diorite and gneisses) origin. The basal till is thin (<1 m) and overlain by weakly developed podzolic or brunisolic soils with a pit-mound terrain. Sphagnum-coniferous bogs are common. Detailed descriptions of the catchment's geology were provided by Jeffries and Snyder (1983), and the overburden was described by LaZerte and Dillon (1984).

Benthic invertebrate assemblages are depauperate in tributary streams flowing into Plastic Lake (Mackay and Kersey 1985) particularly the main inflow (Fig. 1) (LaZerte and Dillon 1984). pH of this inflow varied from 4.3 to 4.9 in spring 1982 (LaZerte 1984). Higher densities and diversities of benthic invertebrates are found below the Plastic Lake outflow (D. Giberson, Freshwater Institute, Winnipeg, Manitoba, pers. comm.).

Plastic Lake outflow (POF) with a pH range of 5.6–6.4 was chosen for this study because it is representative of outflow streams in the area (Fig. 1). The outflow drains into Lake St. Nora 1.4 km downstream. The section used for experimental acidification (msl: 335 m) is located approximately 1.1 km downstream from the POF stream gauging station (msl: 412 m). Riparian vegetation in the study area consists of second growth mixed deciduous-coniferous forest with 70-yr-old white birch (40%), white pine (30%), sugar maple (20%), and poplar and aspen (10%), averaging 23 m in height (Frank Lozano, Forestry School, University of Toronto, Toronto, Ont., pers. comm.). The stream channel in the study area is V-shaped and cut to the sandy till. Large angular to sub-angular boulders with coarse sand interspersed around them are scattered on the stream bottom. Bryophytes grow on top of large boulders and are exposed to stream water only during peak spring melt or during intense summer and fall freshets. Species richness of mayflies in Plastic outflow (5 species) was similar to that in other inflows and outflows (2–6 species) of similar size (D. Giberson, Freshwater Institute, Winnipeg, Man., pers. comm.) and pH (Jeffries et al. 1979) in the Muskoka–Haliburton districts. During the study, water temperature ranged from 13–17.5°C ($\bar{x}$ ± SE, N: 15.3 ± 0.26, 27) in spring and 7–13°C (10.7 ± 0.26, 38)in fall.

Methods

Experimental Design

Acid additions were conducted in spring (9–19 May) and fall (13–23 September) 1982 (Fig. 1). Sulphuric and hydrochloric

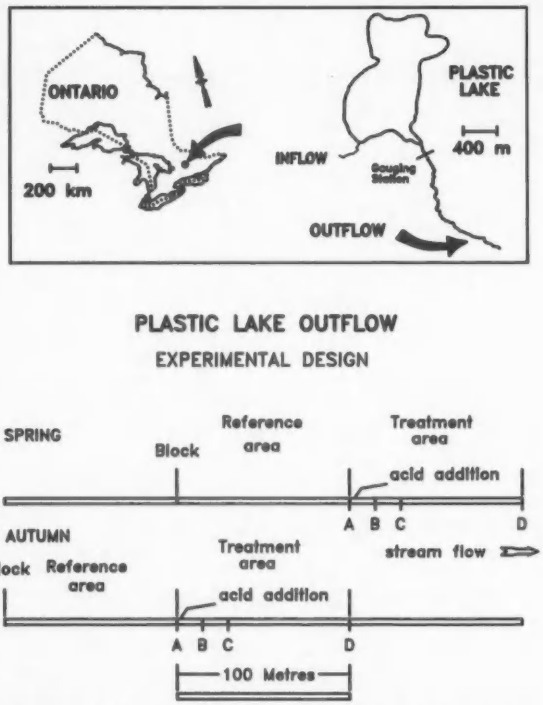

FIG. 1. Plastic Lake outflow (POF) study areas in spring and autumn. Reference area (site A) was 1.5 m above acid addition. Treatment areas (sites B, C, and D) were located 15, 30, and 100 m below acid disturbance. The reference area in spring was the treatment area in autumn.

acids (reagent grade) were mixed and added continuously to the stream to achieve pH depressions similar to those previously reported in the area during snowmelt and rainstorms (Jeffries et al. 1979; Harvey and Lee 1982; B. LaZerte and P. J. Dillon, Dorset Research Centre, Dorset, Ont., unpub. data). Sampling stations were located 1.5 m (site A, reference) above and 15 m (site B), 30 m (site C), and 100 m (site D) below the point of acid addition (Fig. 1). On Day 1, pH was reduced within 2 h from 5.8 in spring and 6.4 in fall to 4.8, and maintained at this level for 4 d (Fig. 2). The pH was then further reduced for 4 d in the same area to 4.2 during both seasons. Thus, acid was added continuously for a total of 8 d. pH was monitored 12 m below the addition every 2 min to 4 h depending upon water flow. Discharge above site A and below site C was determined with a Teledyne Gurley meter at 12-h intervals for the duration of the experiment. Temperature of the stream water was measured at 6–12 h intervals above and below the acid disturbance. The stream reaches used in the spring and fall studies were selected because they were similar in substrate composition. The experimental design in fall was the same as in spring except that the study was done 100 m upstream (Fig. 1). To reduce variability due to site differences, experiments were designed to include partial overlap of stream reaches; i.e. the reference area in spring was the treatment area in fall. Sampling for chemistry, invertebrate drift and benthic communities occurred above and below the point of acid addition as described below.

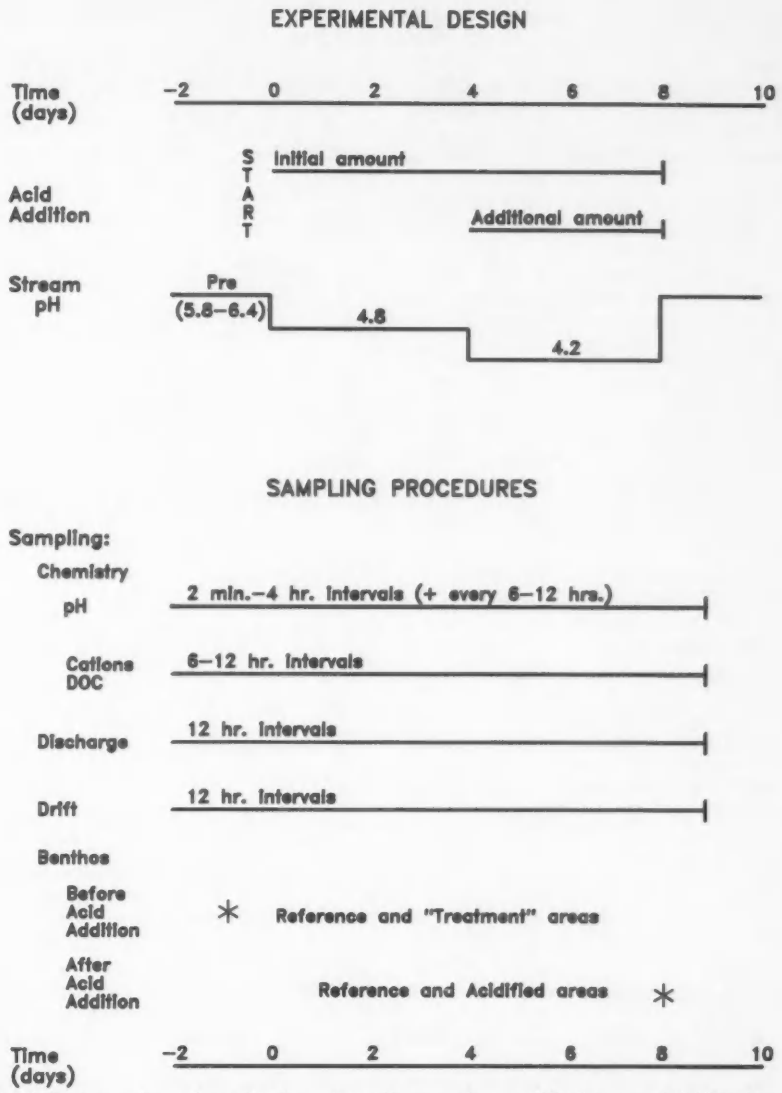

FIG. 2. Summary of experimental design and sampling procedures used for the seasonal pH disturbance experiments.

Water Chemistry

Water samples for chemical analyses were collected at all stations (A–D) every 6–12 h for 2 d prior to and during 8 d of continuous acid addition (Fig. 2). One 250-mL brown polyethylene and two 550-mL clear polystyrene bottles were used to collect each set of water samples at each site. Care was taken not to disturb stream sediments during sampling. Calcium, Fe, Mn, and dissolved organic carbon (DOC) were determined from one 500-mL sample; total Al, Mn, and Zn were determined from the remaining 500-mL. pH was analyzed from water in the 250-mL nalgene bottle with a combination glass electrode standardized against appropriate buffers. pH measurements were taken in the field at 2-min to 4-h intervals and within a few hours after transfer to the laboratory, whereas other chemical parameters were determined within a week to 10 d. Cations were analyzed by flame atomic absorption. Dissolved organic carbon (DOC) concentrations were determined using a colorimetric procedure after UV (ultraviolet) digestion. Inorganic monomeric Al (IMA) was estimated by procedures reported by LaZerte (1984).

Drift Studies

Invertebrate drift was monitored above (site A) and below (sites B and C) the point of acid addition. Drift nets were placed side by side, extending from one stream bank to the other at three sampling stations (sites A, B, and C; Fig. 1). Each net was constructed of a polyvinylchloride frame (15 × 30 cm) to

which was attached a collecting bag (1 m long) made of Nitex (mesh opening 253 μm). A rectangular 4.8- × 4.8-cm wooden frame (~2 m wide × 0.5 m high) covered with Nitex screen (273-μm mesh) blocked the stream 100 m above acid addition so that equal areas above and below the disturbance were studied (Fig. 1). A similar block was positioned 2 m below the acid drip point within the mixing zone to ensure that organisms exposed to pH levels below 4.8 or 4.2 were not collected in drift nets at site B. Drift nets were removed and immediately replaced with clean ones every 12 h, 2 d before (-2 and -1, Fig. 2) and 8 d during experimental pH depressions (4 d at pH 4.8, 4 d at pH 4.2, Fig. 2). At each station, contents of all drift nets extending from bank to bank from the two 12-h periods were combined for each day. Drift samples were preserved in 10% formalin. Numbers of individuals collected were converted to number $\cdot 100\ m^{-3} \cdot d^{-1}$.

Benthic Studies

A cylindrical core sampler (area of 0.0314 m^2) was used to determine density of invertebrates on the stream bottom. In spring, two habitats were selected based on current flow and sediment type. The first was characterized by fast current flowing over pebbles and sand and the second by slow current flowing over organic debris. In fall, coarse organic debris completely covered pebbles and sand on the stream bottom and the current was slow. During both seasons, benthic samples were collected in 100-m reference and treatment study sections before and after 8 d of acid addition (Fig. 1, 2) via a stratified random design. Ten bottom samples were collected from the fast current and five from the slow current habitat in spring and 15 from the slow current habitat in fall, both before and after acid addition in reference and treatment areas. The core sampler was placed into the substrate to a depth ranging from 0 to 15 cm. Stream sediment was mixed vigorously, and organisms suspended in water inside the core were removed with a hand net. This procedure was repeated until no organisms were collected. Surface sediments (~1 cm) in each core sample were also collected after the mixing and collecting process to ensure that caddisflies and molluscs were taken. Invertebrates were preserved in 10% formalin.

Subsampling Procedures for Biological Samples

In the laboratory, drift and bottom samples were washed through an elutriator (Stewart 1975), and cased caddisflies were picked from the residual gravel. Both drift net collections and bottom samples from each station were thoroughly mixed and then washed through a series of graduated sieves (0.6–0.01-mm aperture size) to remove larger pieces of organic debris. Organisms retained by the 0.6-mm mesh were counted individually. Finer screenings were picked over by hand, and any larger organisms were removed and counted. The remaining fine materials were mixed in 1 L of water and allowed to settle slowly in a sieve with a mesh size of 0.01 mm. When the material had drained thoroughly, it was divided into quarters with a cross-shaped partition made to fit snugly into the sieve, and one quarter was examined microscopically (10×). If necessary, an additional one quarter subsample was taken by the same method.

To determine if subsamples adequately represented whole samples, five drift and five benthic samples were randomly chosen and then each sample was divided into quarters as outlined above, and each quarter was subsampled at least five times. Nine out of the ten samples had mean subsample counts within the 95% C.I. for mean totals; one sample had three out of five subsamples within the same intervals. Therefore, I assumed that subsample counts fulfilled the criterion of randomness, and subsampling of remaining samples was conducted. Both larger specimens and those in quarter subsamples were identified to genus (see Merritt and Cummins 1984) and counted.

Diversity

Generic diversities were computed for each benthic sample using Brillouin's formula for diversity per individual (H; Pielou 1975). Evenness (J) was also calculated to determine equitability of abundance of genera in each bottom sample. The decision to determine benthic diversity at the generic rather than species level was justified because the genus is thought to represent a valid ecological as well as a morphological type (Wiggins and Mackay 1978; Merritt and Cummins 1984) and because additional information provided by the diversity component at the species level is small relative to the diversity component at the generic level for aquatic insects and fish (Kaesler and Herricks 1979).

Statistical Analyses

A student's *t*-test was used to test for differences in mean chemical concentration and drift density between paired samples (i.e. site A minus B, site A minus C) within a season and a two sample *t*-test for differences between seasons (site A spring vs. site A fall, etc.). Non-metric multidimensional scaling analyses (NMDS, Kruskal 1964) were used to determine what associations existed between stream chemistry, discharge, and invertebrate drift because NMDS is more appropriate for non-linear data sets than other ordination procedures (Kenkel and Orloci 1986). Only the most common taxa, those comprising 95% of the drift, were used in the analyses. Resulting taxa (23 in spring, 19 in fall) were vector normalized (Legendre and Legendre 1983; referred to as stand norm standardization by Kenkel and Orloci 1986). Euclidean distance association matrices (Fasham 1977) were then calculated (vector normalized plus Euclidian distance calculation = chord distance), and the NMDS was run on a microcomputer using SYSTAT MDS routines in 1–3 dimensions (Wilkinson 1988). Spearman's rank correlations between NMDS dimension scores and selected environmental variables were computed. Similarly, rank correlations between NMDS scores and taxa in drift samples were calculated to aid in interpretation of invertebrate species patterns in relation to position of samples on each of three axes (Sprules 1980).

Statistical analysis of the benthic study was patterned after the optimal impact study design proposed by Green (1979). Samples were collected at sites A and B before short-term pH disturbance and at the same two locations 8 d after pH disturbance, with pH reduction occurring at site B only. Benthic data were transformed using log $(x + 1)$ to remove observed heterogeneity of error variances. I used a two-way (areas-by-times) factorial ANOVA or MANOVA design to test the null hypothesis that no change in benthic communities (total numbers, numbers within behaviourial, functional, and taxonomic groups or numbers within genera) occurred in the pH-disturbed area against the hypothesis of no areas-by-times interaction. A significant interaction between space and time, indicating that a difference in communities between sites was present after acid

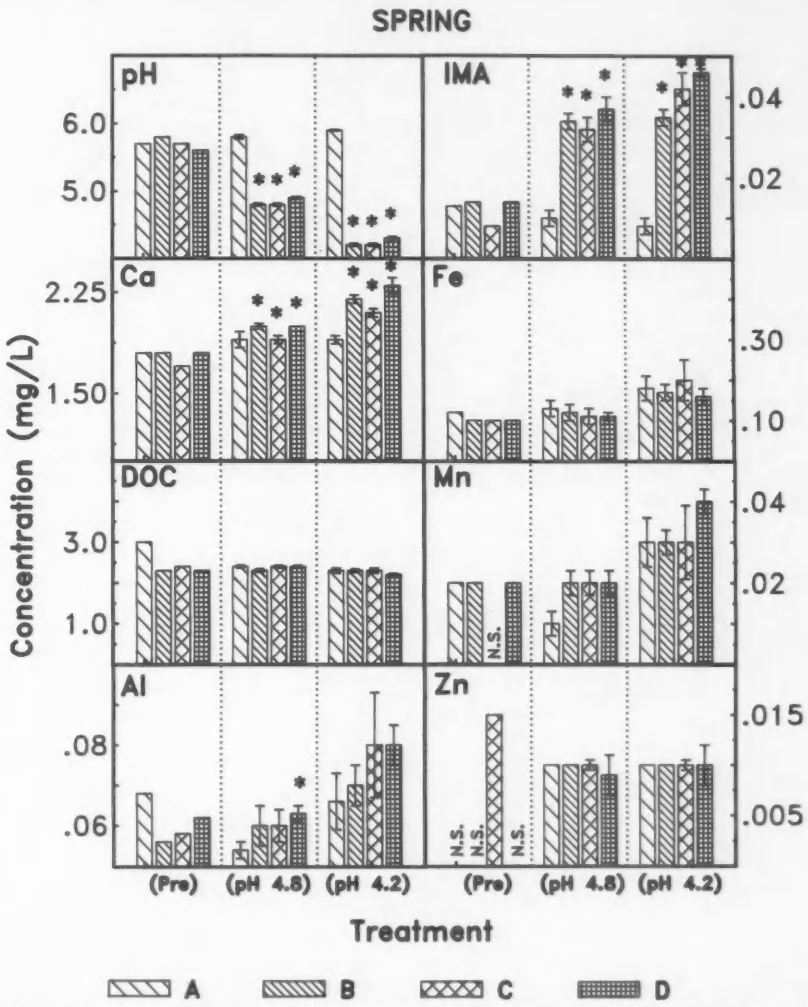

FIG. 3. Daily mean changes (± SE, $N = 8$) in water chemistry in spring prior to and during acid disturbance in POF. Site A (reference area) was located 1.5 m above and sites B, C, and D 15, 30, and 100 m, respectively, below acid addition. No vertical error bars are indicated for (Pre) data because only one sample was taken for each site. N.S. = no sample collected. Pre = samples collected at all sites prior to manipulation. pH 4.8 = reduction of pH at sites B, C, and D to 4.8. pH 4.2 = reduction of pH at the same sites to 4.2. IMA = inorganic monomeric aluminum. Asterisks represent $P < 0.05$, paired t-test.

addition, was detected using either a univariate or a Wilks' Lambda multivariate F-statistic (see Results). If an interaction was demonstrated, I then used a one-way ANOVA or MANOVA for each time (before and after) to determine what differences in benthic communities occurred in the two study areas (reference and treatment) before pH disturbance and how these differences between the same two areas changed after the pH manipulation. I used the two-way factorial MANOVA on the 10 most abundant genera of Diptera from the fast-current habitat in spring because this order had the greatest numerical abundance and because only benthic density of Diptera was significantly reduced in the acid-disturbed area (see Results). Pseudoreplication was a problem with this study; thus, differences between reference and treatment areas may not be attributed solely to treatment effects (Hurlbert 1984).

Results

Stream Chemistry and Hydrology

During experimental pH depression to 4.8, water chemistry in spring and fall reference areas (A sites) differed significantly (two sample t-test, $P < 0.05$) in pH, DOC, inorganic monomeric aluminum (IMA), Ca, and Mn (Fig. 3, 4). During experimental pH depression to 4.2, spring and fall reference areas differed significantly only in pH and concentrations of

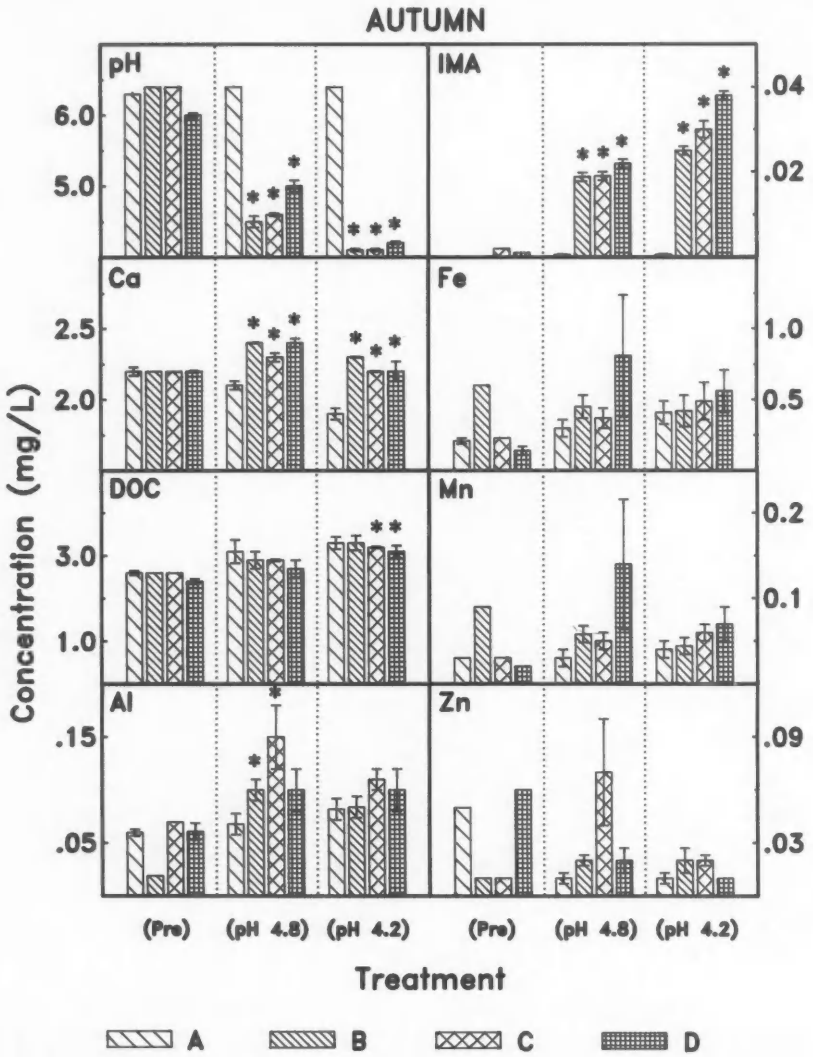

FIG. 4. Daily mean changes (± SE, N = 3 to 8) in water chemistry in fall prior to and during acid disturbance in POF. See Fig. 3 legend for details.

DOC and IMA. Variations in Ca and Mn were not significantly different ($P > 0.05$) when pH was reduced to 4.2, possibly due to fluctuations in discharge (Fig. 5).

Changes in DOC concentration were more closely associated with the hydrologic than the chemical regime. Dissolved organic carbon concentrations were lower in spring when discharge was high and higher in fall when discharge was low. In spring, discharge in general decreased (but not to the low fall level) during pH 4.8 and 4.2 experiments (Fig. 5) and was non-scouring based on the small amount of mineral sediments in the drift nets. There was little change in daily concentration of DOC at site A during the entire spring experiment (site A, pH 4.8 – $\bar{x}$ ± SE, N; 2.4 mg DOC/L ± 0.03, 11; site A, pH 4.2 – 2.3 mg DOC/L ± 0.04, 12). In fall, two large increases in discharge were the result of a rainstorm on Day 4 and the collapse of a beaver dam on Day 7, both events resulting in more mineral sediments in the drift nets (J. Findeis, Ontario Ministry of the Environment, Dorset, Ont., pers. obser.). Dissolved organic carbon concentration at site A during the rain storm increased by 54% ($\bar{x}$ ± SE, N; 2.8 ± 0.2, 7 before and 4.3 mg DOC/L during the storm) and following breakage of the beaver dam by 35% (3.1 ± 0.08, 7 before and 4.2 mg DOC/L during) and then dropped in concentration (3.5 mg DOC/L) following rapid increases in discharge (see Findeis and Coleman–Taylor 1988).

During both pH depressions within each season, significant increases in Ca and IMA (paired t-test, $P < 0.05$) occurred at sites B–D relative to site A (Fig. 3, 4). Significant increases in total Al were detected at site D, pH 4.8 in spring, and sites B and C, pH 4.8 in fall, and significant decreases in DOC

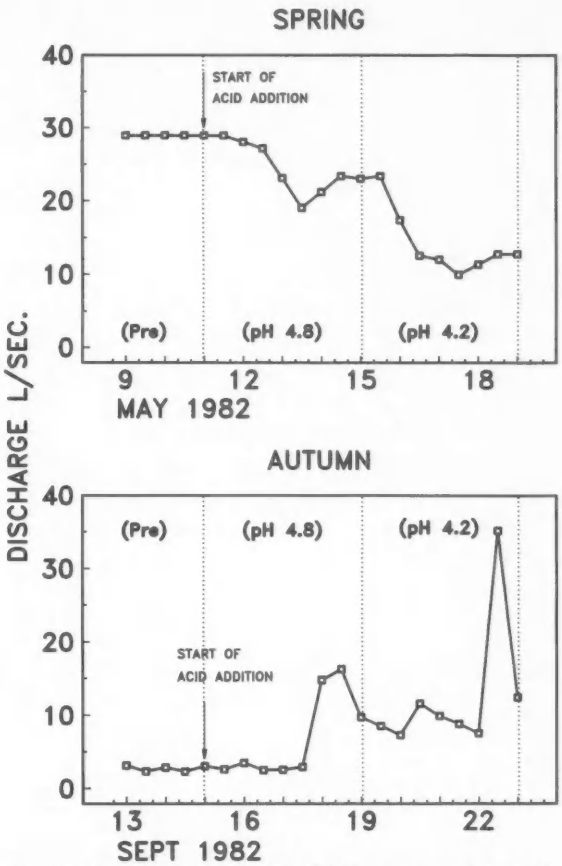

FIG. 5. Mean daily discharge values (L/s) for spring and fall acidification experiments. Pre = prior to manipulation. pH 4.8 for 4 d = addition of acid to pH 4.8. pH 4.2 = addition of acid to pH 4.2 for 4 d.

($P < 0.05$) at C and D, pH 4.2 in fall. No significant differences in concentration above or below the pH depressions in either spring or fall were produced for Fe, Mn, and Zn.

Drift

Site A (reference) had similar drift densities in spring ($\bar{x} \pm$ SE; 220.5 ± 54) and fall (262.3 ± 33.3) for the 8-d experiments. Acid disturbance caused a significant ($P < 0.05$) increase in drift relative to the reference area in fall (site B: 553.3 ± 83; site C: 521.1 ± 91.4), but not in spring (site B: 221.5 ± 47; site C: 321.9 ± 72.9), and more functional groups were affected during the fall experiment. However, in spring significant increases in drift ($P < 0.05$, Fig. 6) occurred for ephemeropterans, dipterans, and miscellaneous groups whereas in fall dipterans, trichopterans, and lepidopterans were significantly affected. Functional groups in spring that responded significantly ($P < 0.05$) to increased acidity by drifting included collector-filterers (collector-gatherers decreased); in fall those that responded were collector-filterers, collector-gatherers, scrapers, and shredders. Behaviourial groups in spring that responded significantly ($P < 0.05$) by drifting included clingers

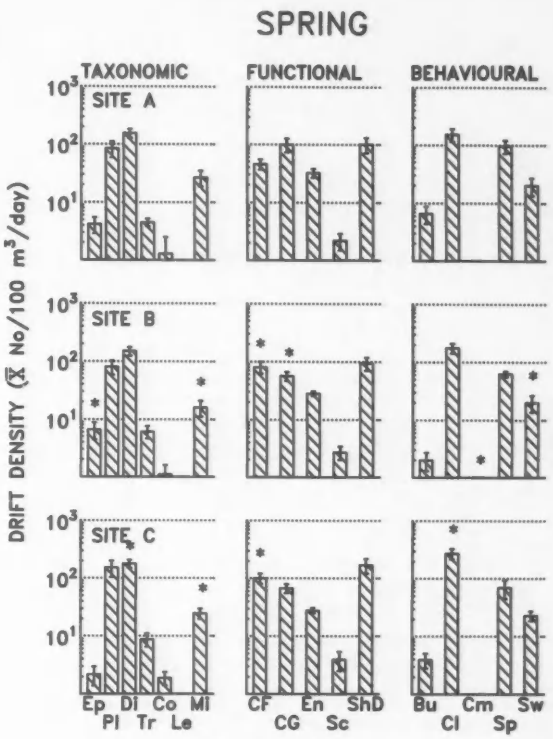

FIG. 6. Drift density ($\bar{x}$ number·100 m^{-3}·d^{-1} ± SE, $N = 8$) at site A (1.5 m above) and B (15 m) and C (30 m) below acid addition in spring by orders (Ep = Ephemeroptera, Pl = Plecoptera, Di = Diptera, Tr = Trichoptera, Mi = miscellaneous), functional group (CF = collector-filters, CG = collector-gatherers, En = engulfers, Sc = scrapers, ShD = shredders) and behaviourial group (Bu = burrowers, Cl = clingers, Cm = climbers, Sp = sprawlers, Sw = swimmers). Asterisks = $P < 0.05$, paired t-test.

TABLE 1. Spearman's rank correlation of spring and autumn invertebrate drift samples (number·100 m^{-3}·day^{-1}) and abiotic variables at sites A, B, and C with NMDS axes for Plastic Lake outflow acidification. Asterisks represent significant correlations (** = 99%, * = 95%).

	Spring		Autumn		
Chemical and physical parameters	Axis 1	Axis 2	Axis 1	Axis 2	Axis 3
pH	0.19	0.57**	0.05	−0.18	0.60**
Discharge	−0.55**	0.51**	0.27	0.06	−0.58**
DOC	−0.26	0.28	0.45**	−0.14	−0.58**
Ca	0.10	−0.75**	−0.62**	0.26	−0.25
Al	0.04	−0.36*	−0.03	−0.17	−0.25
Fe	0.38*	−0.45*	0.26	−0.11	−0.39*
Mn	0.29	−0.50**	0.03	−0.07	−0.21
Zn	0.06	−0.80	—	—	—

and swimmers (climbers decreased); in fall those that responded included climbers and sprawlers.

Two dimensions sufficiently described drift patterns in spring in the NMDS ordination. Although the stress value (0.11) was higher than that recommended by Kruskal (1964), little additional information about correlations among invertebrate taxa, environmental data, and dimension scores was gained by the three-dimensional solution (stress = 0.05). Stress is defined as a "goodness of fit" in a monotonic regression between dissimilarities and the distances. Scores on the first dimension were positively correlated with Fe ($P < 0.05$) and negatively correlated with discharge ($P < 0.01$ (Table 1). On Dimension 2 in spring, scores were positively correlated with pH and discharge ($P < 0.01$) and negatively correlated with Ca ($P < 0.01$), Fe ($P < 0.01$), Al ($P < 0.05$), and Mn ($P < 0.05$ (Table 1).

In fall (three-dimensional NMDS solution, stress = 0.06) scores on the first dimension were positively correlated with DOC ($P < 0.01$) and negatively correlated with Ca ($P < 0.01$), whereas no strong correlations were found on the second dimension (Table 1). Scores on the third axis were positively correlated with pH ($P < .01$) and negatively correlated with discharge ($P < 0.01$), DOC ($P < 0.01$), and Fe ($P < 0.05$).

Many species collected in the drift during spring were correlated more strongly with discharge than pH (Table 1 and 2). Specifically, lower scores on Dimension 1 (i.e. lower discharge) were correlated with significant decreasing drift of *Leuctra, Nemoura, Paramerina, Thienemanniella, Cricotopus* larvae, *Pseudosmittia, Simulium, Ectemnia*, and *Acarina*, but increasing drift of *Allocapnia*, Chironomini, and *Cricotopus* pupae (Table 2). With higher scores on Dimension 2 (i.e. higher pH, higher discharge), *Paramerina, Thienemannimyia, Polypedilum, Tanytarsus, Thienemanniella, Psectrocladius*, and *Ectemnia* drifted more. During lower discharge and lower pH, drift densities increased for *Allocapnia* (stonefly), Harpacticoidea (zooplanktor), Chironomini (midge), *Amphinemura* (stonefly) and *Amelitus* (mayfly). Thus, these latter five taxa displayed acid-sensitivity by increasing in drift density during increased acidity.

Scores on Dimension 1 in fall were positively correlated with DOC (Table 1) and drift densities of *Cricotopus* and *Eukiefferiella* (Table 2) and negatively correlated with Ca (Table 1) and drift densities of *Thienemanniella, Corynoneura*, Oligochaeta, *Dolophilodes*, Nematoda, and *Parametriocnemus* (Table 2). No significant rank correlations existed between Dimension 2 scores and environmental variables in fall (Table 2). Scores on axis 3 were high when drift densities of *Corynoneura* larvae and nematodes were high. During low pH, drift of *Cricotopus* (chironomid), *Eukiefferiella* (chironomid), *Paraleptophlebia* (mayfly) and *Dolophilodes* (caddisfly)

TABLE 2. Spearman's rank correlation of spring and autumn invertebrate drift taxa (number·100 m^{-3}·day^{-1}) at sites A, B, and C with NMDS axes for Plastic Lake outflow acidification. Asterisks represent significance levels (** = 99%, * = 95%). Life stage: N = nymph, P = pupae, L = larvae. Functional group: CG = collector-gatherers, Sh = shredders, En = engulfers, CF = collector-filterers. Behaviourial group: SW = swimmers, Cl = clingers, Sp = sprawlers, Bu = burrowers.

Spring				Autumn				
Taxa	Lifestage/ functional and behaviourial groups	Axis 1	Axis 2	Taxa	Lifestage/ functional and behaviourial groups	Axis 1	Axis 2	Axis 3
Ephemeroptera				Ephemeroptera				
Amelitus	N/CG/SW	0.25	−0.39*	*Ephemerella*	N/CG/SW	0.09	−0.12	−0.19
Plecoptera				*Paraleptophlebia*	N/CG/SW	−0.10	0.12	−0.55***
Leuctra	N/Sh/Cl	−0.63**	−0.09	Diptera				
Nemoura	N/Sh/Cl	−0.46*	−0.15	*Thienemannimyia*	L/En/Sp	−0.17	0.19	−0.32
Amphinemura	N/Sh/Sp	0.19	−0.49**	*Polypedilum*	L/Sh/Cl	−0.21	0.07	−0.03
Allocapnia	N/Sh/Cl	0.42*	−0.69**	*Tanytarsus*	L/CG/Bu	−0.22	0.43*	0.04
Diptera				*Thienemaniella*	L/CG/Sp	−0.67**	0.43*	0.12
Pentaneurini	P/EN/Sp	0.31	−0.23	*Corynoneura*	L/CG/Sp	−0.59**	0.07	0.59**
Paramerina	L/En/Sp	−0.45	−0.52**	*Cricotopus*	L/Sh/Cl	0.42*	−0.12	−0.72**
Thienemannimyia	L/En/Sp	−0.08	0.53**	*Eukiefferiella*	L/CG/Sp	0.38*	−0.12	0.53**
Chironomini	P/CG/Bu	0.53*	−0.58**	*Parametriocnemus*	L/CG/Sp	−0.43*	−0.03	0.07
Polypedilum	L/Sh/Cl	−0.33	0.46**	*Simulium*	L/CG/Cl	−0.34	0.18	0.10
Tanytarsus	L/CG/Bu	−0.08	0.38**	*Atherix*	L/CF/Cl	−0.12	0.08	0.09
Thienemanniella	L/CG/Sp	−0.55**	0.42*	Trichoptera				
Orthocladini	P/CG/Sp	−0.13	−0.02	*Dolophilodes*	L/CF/Cl	−0.49**	0.11	−0.52**
Cricotopus	P/Sh/Cl	0.95**	0.06	*Diplectrona*	L/CF/Cl	−0.30	0.05	.0.05
Cricotopus	L/Sh/Cl	−0.60**	0.19	*Glossosoma*	L/Sc/Cl	0.03	0.20	0.04
Parametriocnemus	L/CG/Sp	−0.16	−0.06	Miscellaneous				
Psectrocladius	L/CG/Sp	−0.30	0.45*	Nematoda	CG/Cl	−0.48**	−0.25	0.58**
Pseudosmittia	L/CF/Sp	−0.59**	0.26	Oligochaeta	CG/Bu	−0.50**	0.28	0.07
Rheocricotopus	L/CF/Cl	−0.26	0.22	Acarina	CG/SW	−0.16	−0.08	−0.05
Simulium	L/CF/Cl	−0.89**	−0.05	Harpacticoida	CG/SW	0.04	0.004	0.08
Ectemnia	L/CF/Cl	−0.54*	0.43*					
Miscellaneous								
Harpacticoida	CG/Sw	0.16	−0.63**					
Acarina	CG/Sw	−0.56**	0.15					

TABLE 3. Mean number (± SE) of individuals (per m^2), number of genera (generic richness), generic diversity (H), and evenness (J) of invertebrates collected in spring (10–18 May) and autumn (12–20 September) with a 0.0314-m^2 benthic cylinder sampler. Asterisks represent $P < 0.05$ (two-way ANOVA).

	Spring							
	Fast current — pebble and sand				Slow current — organic debris			
	Before acid		After acid		Before acid		After acid	
	Reference	Treatment	Reference	Treatment	Reference	Treatment	Reference	Treatment
Total number of individuals	7,046.88 (696.4)	6,655.74 (976.3)	8,026.32 (1,163.9)	3,262.68* (194)	21,166 (4,865.4)	23,907.24 (3,021)	52,927.92 (6,560.97)	40,341.48 (4,621.8)
Number of genera	24.2 (1.2)	22.3 (2.4)	24.9 (1.6)	18.9* (1.0)	14 (2.2)	11.2 (2.4)	20 (2.1)	11.6 (2.5)
Diversity (H)	3.2 (0.08)	3.0 (0.21)	3.0 (0.16)	2.8 (0.095)	2.3 (0.32)	1.6 (0.20)	2.6 (0.17)	2.0 (0.32)
Evenness (J)	0.75 (0.02)	0.82 (0.03)	0.70 (0.03)	0.74 (0.02)	0.63 (0.06)	0.68 (0.04)	0.61 (0.03)	0.59 (0.08)
N	10	10	10	10	5	5	5	5

	Fall			
	Slow current — pebble, sand and organic debris			
	Before acid		After acid	
	Reference	Treatment	Reference	Treatment
Total number of individuals	50,186 (8,186)	86,990 (9,292.2)	85,853.8 (14,649)	146,062 (27,423)
Number of genera	14.5 (1.2)	23.6* (1.0)	22.8 (1.31)	16.5* (0.84)
Diversity (H)	2.4 (0.11)	2.2 (0.12)	2.4 (0.06)	2.4 (0.10)
Evenness (J)	0.66 (0.04)	0.49* (0.03)	0.55 (0.02)	0.61 (0.03)
N	15	15	15	15

increased, and thus these last four species could be considered sensitive to the episodic acid stress.

Benthic Invertebrates

In spring in the fast current, a significant interaction ($P < 0.05$, two-way ANOVA) occurred between total benthic density at sites A and B before and after acidification (Table 3), indicating that variability between reference (A) and treatment (B) sites after pH disturbance was different from variability before. One-way ANOVA showed no differences in benthic density ($P > 0.05$) between sites A and B in spring in the fast current before acid addition; however, significant ($P < 0.05$, one-way ANOVA) reductions in density were evident after acid addition at site B relative to site A. No significant interaction ($P > 0.05$) occurred in spring in the slow current between total benthic densities at sites A and B before and after acidification.

In fall, an interaction ($P < 0.05$, two-way ANOVA) occurred between total benthic densities at sites A and B before and after acidification (Table 3). Pretreatment benthic densities were not significantly different ($P < 0.05$, one-way ANOVA) at reference and treatment sites, and thus no assessment of reduced pH treatment could be made. Although total benthic densities in fall showed no significant response to decreased pH, certain groups were affected. Increased densities in the acidified reach were particularly evident for Chironominae (Tribes: Chironomini and Tanytarsini, probably *Tanytarsus*). It was difficult to identify some genera because they were very small (first and second instars). Other Orthocladinae (*Thienemanniella, Eukiefferiella, Parametriocnemus, Psectrocladius*), however, decreased in density in the same area. Responses of caddisflies (Trichoptera) to pH disturbance varied from increased density for *Dolophilodes* and decreased density for *Hydroptila*.

In spring a significant ($P < 0.05$, two-way ANOVA) interaction in the fast current before and after acidification was found for generic richness, but not for diversity or evenness (Table 3). A one-way ANOVA indicated significant differences ($P < 0.05$) after acidification for generic richness, indicating an effect associated with low pH. No interactions were found in the slow current habitat in spring. In the slow current habitat in fall, significant interaction for generic richness was explained by differences both before and after acidification, whereas significant interaction ($P < 0.05$) for evenness was explained only by differences before acidification. No interaction was determined for benthic diversity in fall.

Partitioning benthic invertebrates into taxonomic, functional, and behaviourial categories revealed that significant interactions ($P < 0.05$, two-way ANOVA) and decreases in density ($P < 0.05$, one-way ANOVA) after acidification resulted for dipterans (Fig. 8) in the fast current during spring and for ephemeropterans (Fig. 9) in fall. Decreases relative to the reference area in the spring fast current were due primarily to those organisms that have the habits of clingers or sprawlers ($P < 0.05$, one-way ANOVA). No significant interactions ($P > 0.05$, two-way ANOVA) were observed for functional guilds in the spring fast current, but fall data showed significant ($P < 0.05$, two-way ANOVA; one-way ANOVA) decreases in density for scrapers after acidification. No interactions

TABLE 4. Mean (± SE) benthic densities (number·m^{-2}) of the dominant Diptera collected at two locations (reference and treatment areas) in spring in mineral sediments (fast current over pebbles and sand) before and after pH disturbance. Asterisks = $P < 0.05$, one-way ANOVA's for before and after acid additions.

	Before acid		After acid	
Taxa	Reference	Treatment	Reference	Treatment
Cricotopus	167 (92 to 301)	269.8 (219 to 332)	356 (276 to 460)	142 (129 to 156)*
Parametriocnemus	1.4 (0 to 2.0)	0 —	97 (55 to 172)	2.2 (1.3 to 3.7)*
Psectrocladius	14.7 (8 to 27)	2.4 (1.3 to 4.3)*	9.9 (4.5 to 22)	4.6 (2.4 to 8.7)
Paramerina	36.5 (20 to 68)	3.4 (1.8 to 6.5)*	9.9 (4.5 to 22)	2.0 (1.3 to 3.2)
Procladius	9.7 (5.2 to 18)	3.26 (1.8 to 6.0)	3.0 (16.5 to 55)	59 (45 to 7.7)
Tanytarsus	509 (435 to 596)	22 (9.2 to 53)*	238 (198 to 285)	140 (117 to 168)*
Thienemanniella	333 (258 to 429)	5.7 (2.3 to 14)*	213 (185 to 245)	4.6 (2.4 to 8.6)
Corynoneura	9.3 (5.0 to 17)	7.6 (3.8 to 15)	1.5 (1.1 to 2.1)	0 —
Polypedilum	87.0 (65 to 117)	39 (20 to 74.7)	11.0 (5.6 to 22)	5.0 (2.5 to 9.7)
Prosimulium	5.65(2.7 to 11.7)	5.6 (2.7 to 11.6)	205 (89 to 473)	1.4 (1.0 to 2.0)*
Simulium	16.3 (6.2 to 43)	21.8 (11.0 to 44.3)	159 (75 to 338)	4.6 (2.4 to 8.7)*

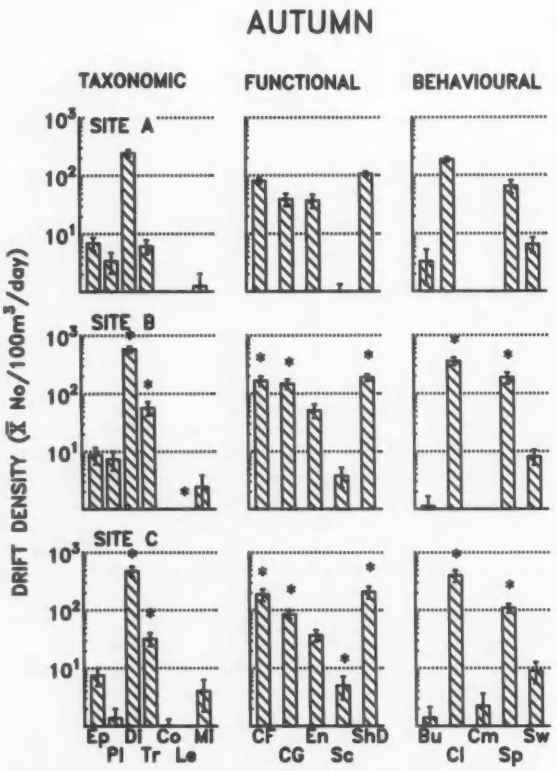

FIG. 7. Drift density ($\bar{x}$ number·100 m^{-3}·d^{-1}) in autumn. See Fig. 6 for taxonomic, functional and behaviourial group legends.

($P > 0.05$) for any guild category were found for spring data from the slow current habitat.

A significant interaction (Wilks' Lambda = 0.43; F-approx. = 3.2; df = 11,26; $P < 0.007$) between space and time occurred for the dominant dipterans (Table 4) in benthic samples collected in the fast current in spring (Table 3). Significant decreases in density ($P < 0.05$, univariate F test) occurred in the treatment area relative to the reference section for *Tanytarsus* and *Thienemanniella* before and after acidification, and thus decreases could not be correlated with pH disturbance. Similarly, significant decreases in *Psectrocladius* and *Paramerina* in the treatment area before manipulation did not result from variations in acidity. However, significant reductions ($P < .05$, univariate F test) in density after acidification in the treatment area for *Cricotopus*, *Parametriocnemus*, *Prosimulium* and *Simulium* genera were associated with pH disturbance.

A comparison of taxa collected in the drift with those in bottom samples revealed that some species found in drift nets (Table 2, Fig. 6 and 7) were seldom collected from the stream bottom (Table 4, Fig. 8 and 9). In spring, five taxa (stoneflies *Allocapnia* and *Amphineura*, mayfly *Amelitus*, midge tribe Chironomini, and Harpacticoidea) increased in drift during acidification, whereas four different taxa (midges *Cricotopus* and *Parametriocnemus* and blackflies *Prosimulium* and *Simulium*) decreased in the benthos. In fall, four taxa (midges *Cricotopus* and *Eukiefferiella*, mayfly *Paraleptophlebia*, and caddisfly *Dolophilodes*) increased in the drift during acidification. Of these four taxa, only one (*Eukiefferiella*) decreased in the benthos along with four different genera (midges *Thienemanniella*, *Parametriocnemus*, *Psectrocladius*, and caddisfly *Hydroptila*).

Fluctuations in hydrology produced variable responses in drift and benthos in fall. During the rainstorm on day 4 of the acidification, discharge increased more than threefold (Fig. 5), and the number of drifting organisms doubled. Three days later a beaver dam broke, producing a discharge more than twice that during the rainstorm. Drift density, however, did not increase during this time, but benthic densities did (Findeis and Coleman–Taylor 1988).

Discussion

Acid disturbance applied in spring to Plastic Lake outflow did not cause an overall increase in total numbers in drift. This result differs from that found in experimentally acidified streams of similar pH in New Hampshire (Hall et al. 1980b, 1982, 1987), but at both New Hampshire and POF, mayflies increased significantly in the drift in response to increased acidity. In spring, however, the total community composition in drift at POF was strongly associated with discharge and not pH; that is, spring drift decreased with diminishing discharge. Ciborowski (1983) and Hopkins et al. (1989) similarly found positive correlations between discharge and drift. The exceptions in this study (*Allocapnia* nymphs, Chironomini, and *Cri-*

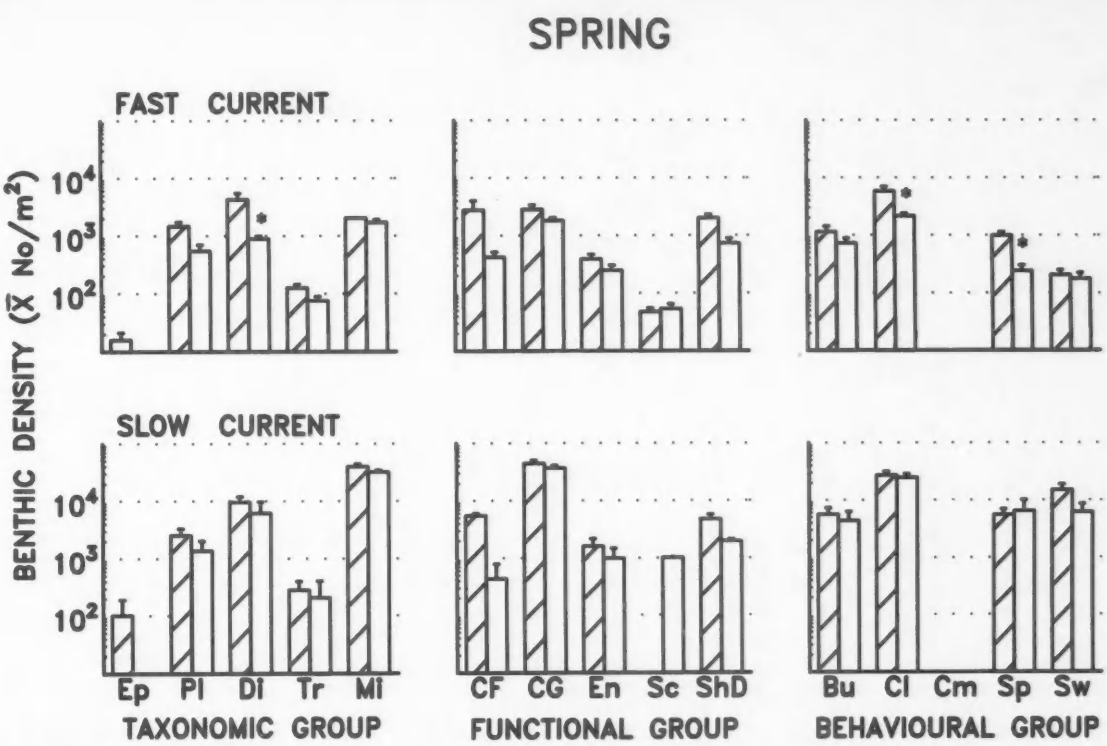

FIG. 8. Benthic density ($\bar{x}$ number/m^2 ± SE, $N = 10$ in fast and five in slow flow habitats) in reference (hatched bar) and treatment areas (clear bar) in fast and slow currents after pH disturbance in spring. See Fig. 6 for legend. Asterisks = $P < 0.05$ (one-way ANOVA).

cotopus pupae) displayed increases in drift density with decreased discharge. This behaviour may have been related to either active swimming responses (Minshall and Winger 1968) or pre-emergence activity of the pupae.

In contrast, the same pH disturbance regime applied to POF in fall was associated with a significant increase in the drift. Compared to spring, organisms were much smaller (Giberson and Hall 1988) and presumably more sensitive to lower pH (Fiance 1978). The mayflies (*Amelitus, Paraleptophlebia*), stoneflies (*Amphinemura* and *Allocapnia*), chironomids (tribe: Chironomini and Orthocladini: genera *Cricotopus* and *Eukiefferiella*) and the caddisfly (*Dolophilodes*) that responded by increased drift at low pH were the same taxa absent in acidic streams (pH <5; Mackay and Kersey 1985; Hall and Ide 1987) or that disappeared in experimentally acidified channels located in other Canadian Shield streams (Allard and Moreau 1987). These taxa reside in the water column (clingers) and swim above or climb and sprawl on the substrate (Merritt and Cummins 1984), and thus episodic pH disturbances would stress these organisms sooner than those that burrow into the substrate (Allard and Moreau 1987).

The total number of benthic invertebrates in spring (with high baseflow discharge compared to fall) decreased in response to short-term, experimentally lowered pH more than twice as much in fast current as in slower flowing habitats. Difference in response between invertebrates in these two habitats may be attributed to deposition of organic matter in the slower current habitat, allowing neutralization of hydrogen ions and increasing pH (Oliver and Kelso 1983). Dermott et al. (1986) reported that total abundance of lake benthic invertebrates was more strongly associated with percent organic content of sediments than with lake pH. Similarly, Fiance (1979) showed that invertebrates found in organic debris were less affected by acidification than those found in mineral sediments and further that some acid-tolerant species (e.g. *Ephemerella funeralis* and *Leuctra* app.) moved from organic to the mineral sediments and filled spaces vacated by acid-sensitive species. In my study, clingers and sprawlers in the benthos were most affected by pH depressions in the mineral sediments of the fast current habitat, paralleling results from drift samples.

Despite decreases in densities of the benthos during spring acidification, overall diversity and evenness did not change in either the fast- or slow-current habitat. Lower diversity and evenness have been reported in many acidic streams (Herricks and Cairns 1974; Simpson et al. 1985; Kimmell et al. 1985), and thus results from the present study were unexpected. Perhaps duration and intensity of the pH disturbance were not great enough to decrease these parameters. Reductions in diversity have been found in streams that were either acidified for extended periods of time (Simpson et al. 1985) or had been pulsed with acid to produce a pH below 4.0 for 15 min. (Herricks and Cairns 1974).

In contrast to spring, total number of benthic invertebrates in fall (when baseline discharge was low) increased significantly over time in both control and treatment sections. Three reasons may explain the observed increase in numbers. First, organisms could have been hatching at this time; first and second instars and egg masses were present in the benthos. Sec-

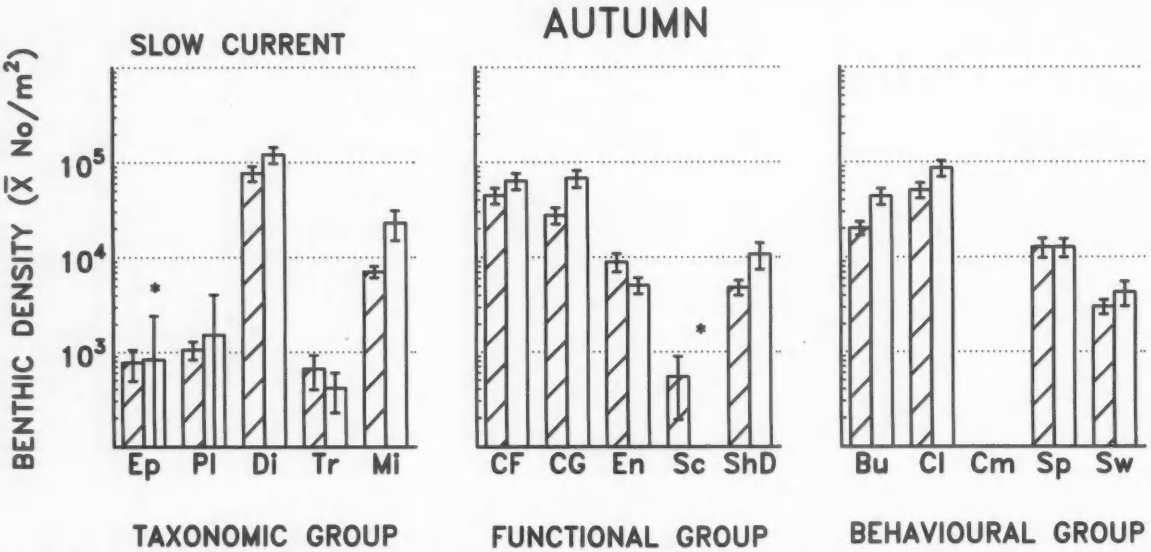

FIG. 9. Benthic density ($\bar{x}$ number/m^2 ± SE, N = 15) in reference (hatched bar) and treatment (clear bar) areas after pH disturbance in autumn. See Fig. 6 for legend. Asterisks = P <0.05 (one-way ANOVA).

ondly, organisms may have already hatched several centimetres below the sediment–water interface (hyporheic zone) and moved to the surface to fill spaces vacated by acid-sensitive Orthocladinae midges and caddisflies. Third, scouring discharge and a possible increased flow in shallow substrates (Munn and Meyer 1988; Giberson and Hall 1988) could have physically forced invertebrates closer to sediment surface. Whatever factor was involved, a direct effect on benthos due to pH was not observed. Schlosser and Ebel (1989) likewise reported dramatic increases in benthic density with elevated flow, although discharge regimes were nonscouring compared to fall flows in POF. In contrast, in a stream in Wales with higher ambient pH (6.6–7.0) than POF (5.6–6.0), experimental acid and aluminum additions in late summer produced increased drift and reduced benthic densities during increased discharge (Weatherley et al. 1988). The observed decrease in Orthocladinae genera in low pH in this study is consistent with results from stream surveys in Muskoka–Haliburton Districts (MacKay and Kersey 1985) and from long-term (3–5 mo) acidification experiments in New Hampshire (Hall et al. 1980b) and Québec (Allard and Moreau 1987). Increases in Chironomini and Tanytarsini tribes (subfamily Chironominae) in acidic streams (<4.5) also have been reported by the same investigators.

Resh et al. (1988) proposed that some level of disturbance is, in part, responsible for maintaining diversity of invertebrates in streams. In this study, chemical (increased acidity) and physical (discharge) disturbance did not significantly alter diversity or evenness within either season. Reice (1985) likewise reported no significant change in diversity during a physical disturbance experiment in a woodland stream in North Carolina. However, at POF, differences did exist in these community measurements between habitats and between seasons. The fast current habitat showed greater diversity than the slow current habitat. During spring when disturbances presumably are more predictable and more frequent, diversity was high; in fall when disturbances are thought to be unpredictable, diversity was low. Robinson and Minshall (1986), however, found higher diversity in fall than summer. The connection between diversity values and frequency and predictability of chemical and physical disturbances obviously is not clear.

An unexpected result at the Plastic Lake outflow manipulation was the low concentration of mobilized Al at pH 4.8 and 4.2 compared to upland streams (Hall et al. 1987) in the same catchments, eliminating Al toxicity as a factor in structuring benthic communities in POF during this study. In Norris Brook, New Hampshire, Al ranged in concentration from 120 to 1200 μg/L at pH 4.0–4.2 in spring (Hall et al. 1987) compared to 54–76 μg/L at POF. Variability among locations may be related to either sediment minerals (Hall et al. 1987) or plant cover (Caines et al. 1985; Tipping and Hopwood, 1988) or some combination of the two. Henriksen et al. (1988) reported that high levels of mobilized Al in an experimentally acidified stream in Norway were correlated with high liverwort biomass. Similar results have been found in the Lake District, northern England (Tipping and Hopwood 1988). Streams that were acidified in New Hampshire and Ontario predominantly have mineral sediments with bryophytes on the tops of larger boulders. These plants are covered only occasionally by stream water at peak snowmelt and during intense rain storms. During POF acidification experiments, stream water level was below that of the semi-aquatic bryophytes. Thus, differences in Al mobilization between New Hampshire and Ontario are believed to be the result of differences in sediment minerals at the two locations. Rate of dissolution of cations was lower at POF than at the upland streams in New Hampshire (Hall et al. 1987). Upland tributary streams have thinner soils and greater fluctuations in discharge and pH, and thus more mineral weathering takes place there than in downstream reaches (Hall et al. 1987), particularly lake outflows. Furthermore, lakes act as sinks and thus impede weathering in their outflow streams.

Increase in DOC concentration in the reference area during rapidly elevated discharge following storms and in fall relative to spring in this study is consistent with results reported by Hall et al. (1980b) and Hall and Likens (1984) in a stream in the

White Mountains, New Hampshire and implies that during storms increased amounts of DOC may be flushed out of the upper soil horizons and into stream water (Reid et al. 1981). However, the influence of elevated H^+ and Al concentration during experimental acid addition on reducing DOC concentrations in POF was more variable than that reported by Hall and Likens (1984). In New Hampshire, reductions in DOC in the stream as a result of experimental acid addition occurred in spring but not autumn. In contrast, POF concentrations of DOC were significantly reduced in fall but not in spring during experimental acid addition. Reasons for these differences are, at this point, not obvious.

This study demonstrated the interrelationship between life histories of benthic invertebrates and disturbances, particularly discharge and acidity. In spring, increased discharge from snowmelt is a predictable annual event, and Reice (1985) suggests that organisms may not perceive it as a disturbance. During that time, many individuals of species that are acid-sensitive may be able to survive by taking refuge in organic, hydrogen ion-neutralizing sections of the stream. In fall, when discharge is low and spates unpredictable, an intense, acidic rainstorm can be accommodated by invertebrate communities because in shallower water (relative to spring) there is more water-sediment contact time during which H^+ neutralization can take place (Hall and Likens 1984), and many benthic insects are in smaller life stages during fall and burrow into hyporheic sediments, thereby obtaining protection from low pH disturbances. Giberson and Hall (1988) found insects in the hyporheic zone in fall, but not spring, in another Canadian Shield lake outflow stream. Furthermore, these insects exist in large numbers, ready to repopulate areas scoured by unpredictable fall rainstorms. Thus, these invertebrates are quite resilient because they have developed life cycle strategies to compensate for episodic physical and chemical disturbances.

One difficulty in quantifying the effect due to pH vs. that due to physical disturbance involved choice of scale (i.e. time or distance interval between sample collections) (Frost et al. 1988). A change in distribution and abundance of benthic invertebrates brought about by scouring of benthic sediments in fall made the original sampling design less appropriate to determine pH effects. Sampling should have been carried out at more frequent intervals (at least every other day) before, during, and after increased discharge to detect the effect of reduced pH on small benthic organisms. An interesting point, however, is that the block placed bank to bank 2 m below the acid addition site prevented organisms scoured out upstream from being deposited downstream. Thus, the large increases in benthic numbers below acid addition likely came from hyporheic sediments.

The intent of this study was to determine the influence of episodic pH disturbances on biogeochemistry and community structure of a lake outflow during different seasons. A dominant pattern that emerged from this investigation was that, although pH was an important influence on a few species, an equally significant factor correlated with fluctuations in water chemistry, invertebrate drift patterns, and benthic densities in this stream reach was the hydrologic regime. In spring when discharge disturbance was less, a significant reduction in benthic numbers after acid addition was detected in riffle habitats. In fall when invertebrates were in smaller life stages and when the stream ecosystem was more physically disturbed by fluctuations in discharge, benthic effects due to pH reductions were either absent or masked. Thus, no evidence was accumulated to support my hypothesis that fall invertebrate communities relative to spring would be more severely affected by reduced pH because organisms are smaller in size.

The extent to which the conclusion about the hydrologic regime can be applied to other streams is not known because lotic environments are spatially heterogeneous. Giberson and Hall (1988) found no changes in benthic densities in another Canadian Shield outflow stream during a spring flood and attributed the results to stability of the substrate. In contrast, in streams studied for the last half-century in Algonquin Provincial Park (Ide 1940; Sprules 1947; Davies 1950; Hall and Ide 1987), physical disturbance was a dominant factor that was associated with emergence patterns of over 100 species of insects, whereas fluctuating pH and associated chemical changes such as Al concentration, although important in regulating the patterns of some mayfly species, were secondary to discharge (R. J. Hall, unpubl. data).

Acknowledgements

I thank B. Clark, K. Clarke, J. Findeis, R. Reid, J. Jones, J. McClennan, T. Pawson, and J. Munro for assistance in the field; A. Nicolls and J. Findeis for computer programs; and Mary Coleman–Taylor for sorting, identifying, and enumerating the benthic invertebrates. R. Bailey and an anonymous reviewer provided helpful comments on an earlier draft.

References

ALLARD, M., AND G. MOREAU. 1987. Effects of experimental acidification on a lotic macroinvertebrate community. Hydrobiologia 144: 37–49.

BURTON, T. M., R. M. STANDFORD, AND J. W. ALLAN. 1982. The effects of acidification on stream ecosystems, p. 209–235. *In* D'Tri[ed.] Acid precipitation: effects on ecological systems. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

CAINES, L. A., A. W. WATT, AND D. E. WELLS. 1985. The uptake and release of some trace metals by aquatic bryophytes in acidified waters in Scotland. Environ. Pollut. (B) 10: 1–18.

CIBOROWSKI, J. J. H. 1983. Influence of current velocity, density, and detritus on drift of two mayfly species (Ephemeroptera). Can. J. Zool. 61: 119–125.

CORBETT, E. S., AND J. A. LYNCH. 1982. Rapid fluctuations in streamflow pH and associated water quality parameters during a stormflow event. International Symposium on Hydrometeorology. Am. Water Res. Assoc. p. 461–464.

DAVIES, D. M. 1950. A study of the blackfly populations of a stream in Algonquin Park, Ontario. Trans. R. Can. Inst. 59: 121–160.

DERMOTT, R., J. R. M. KELSO, AND A. DOUGLAS. 1986. The benthic fauna of 41 acid sensitive headwater lakes in north central Ontario. Water Air Soil Pollut. 28: 283–292.

FASHAM, M. J. R. 1977. A comparison of nonmetric multidimensional scaling, principal components and reciprocal averaging for the ordination of simulated coenoclines, and coenoplanes. Ecology 58: 551–561.

FIANCE, S. B. 1979. Effects of lowered pH on the composition and structure of stream invertebrate communities. Ph.D. thesis, Cornell University, Ithaca, N.Y.

1978. Effects of pH on the biology and distribution of *Ephemerella funeralis* (Ephemeroptera). Oikos 31: 332–339.

FINDEIS, J. G., AND M. COLEMAN–TAYLOR. 1988. Chemical and biological data summary for Plastic Lake outflow acidification experiments (spring and autumn, 1982) Environment Ontario, Data Rep. DR 88/2. 107 p.

FROST, T. M., D. L. DE ANGELIS, S. M. BARTELL, D. J. HALL, AND S. H. HURLBERT. 1988. Scale in the design and interpretation of aquatic community research, p. 229–258. *In* S. R. Carpenter [ed.] Complex interactions in lake communities Springer–Verlag New York, Inc., New York, NY.

GIBERSON, D. J., AND R. J. HALL. 1988. Seasonal variation in faunal distribution within the sediments of a Canadian Shield stream, with emphasis on responses to spring floods. Can. J. Fish. Aquat. Sci. 45: 1994–2002.

GJESSING, E. T., A. HENRIKSEN, M. JOHANNESSEN, AND R. F. WRIGHT. 1976. Effects of acid precipitation on freshwater chemistry, p. 64–85. *In* F. W.

Braekke [ed.] Impact of acid precipitation on forest and freshwater ecosystems in Norway. Res. Rep. 6/76, SNSF Project, Oslo, Norway.

GREEN, R. H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley and Sons, New York, NY. 269 p.

HALL, R. J., R. C. BAILEY, AND J. FINDEIS. 1988. Factors affecting survival and cation concentration in the blackflies *Prosimulium fuscum/mixtum* and the mayfly *Leptophlebia cupida* during spring snowmelt. Can. J. Fish. Aquat. Sci. 45: 2123–2132.

HALL, R. J., C. D. DRISCOLL, AND G. E. LIKENS. 1987. Importance of hydrogen ions and aluminum in regulating the structure and function of stream ecosystems: an experimental test. Freshwater Biol. 18: 17–43.

HALL, R. J., C. D. DRISCOLL, G. E. LIKENS, AND J. M. PRATT. 1985. Physical, chemical, and biological consequences of acid and aluminum additions to a stream. Limno. Oceanogr. 30: 212–220.

HALL, R. J., AND F. P. IDE. 1987. Evidence of acidification effects on stream insect communities in central Ontario between 1937 and 1985. Can. J. Fish. Aquat. Sci. 44: 1652–1657.

HALL, R. J., AND G. E. LIKENS. 1984. Effect of discharge rate on biotic and abiotic chemical flux in an acidified stream. Can. J. Fish. Aquat. Sci. 41: 1132–1138.

HALL, R. J., G. E. LIKENS, S. B. FIANCE, AND G. HENDRY. 1980b. Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. Ecology 61: 976–989.

HALL, R. J., M. J. PRATT, AND G. E. LIKENS. 1982. Effects of experimental acidification on macroinvertebrate drift diversity in a mountain stream. Water Air Soil Pollut. J. 18: 273–287.

HALL, R. J., T. F. WATERS, AND E. F. COOK. 1980a. The role of drift dispersal in the production ecology of a stream mayfly. Ecology 61: 37–43.

HARVEY, H. H., AND C. LEE. 1982. Historical fisheries changes related to surface water pH changes in Canada, p. 45–55. *In* Proc. Int. Conf. Acid Rain/Fisheries. American Fisheries Society, Cornell.

HENRIKSEN, A., B. M. WATHNE, E. J. S. ROGEBERG, S. A. NORTON, AND D. F. BRAKKE. 1988. The role of stream substrates in aluminum mobility and acid neutralization. Water Res. 22: 1069–1073.

HERRICKS, E. E., AND J. CAIRNS. 1974. The recovery of stream macrobenthos from low pH stress. Revista de Biologia (10): 1–11.

HOPKINS, P. S., K. W. KRATZ, AND S. D. COOPER. 1989. Effects of an experimental acid pulse on invertebrates in a high altitude Sierra Nevada stream. Hydrobiologia 171: 45–58.

HURLBERT, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187–211.

IDE, F. P. 1940. Quantitative determination of the insect fauna of rapid water. Univ. Toronto Stud. Biol. Ser. 47, Publ. Ont. Fish. Res. Lab. LIX.

JEFFRIES, D., C. COX, AND P. J. DILLON. 1979. Depression of pH in lakes and streams in central Ontario during snowmelt. J. Fish. Res. Board Can. 36: 640–646.

JEFFRIES, D. S., AND W. R. SNYDER. 1983. Geology and geochemistry of the Muskoka–Haliburton study area. Ontario Ministry of the Environment, Dorset, Ont. Data Rep. DR 83/2. 101 p.

KAESLER, R. L., AND E. E. HERRICKS. 1979. Hierarchical diversity of communities of aquatic insects and fish. Water Resour. Bull. 15: 1117–1125.

KELLER, W. 1983. Spring pH and alkalinity depressions in Lake Superior tributaries. J. Great Lakes. Res. 9: 425–429.

KENKEL, N. C., AND L. ORLOCI. 1986. Applying metric and nonmetric multidimensional scaling to ecological studies: some new results. Ecology 67: 919–928.

KIMMEL, W. G., D. J. MURPHY, W. E. SHARPE, AND D. R. DEWALLE. 1985. Macroinvertebrate community structure and detritus processing rates in two southwestern Pennsylvania streams acidified by atmospheric deposition. Hydrobiologia 124: 97–102.

KRUSKAL, J. B. 1964. Multidimensional scaling by optimising goodness to fit to a nonmetric hypothesis. Psychometrika 29: 1–27.

LAZERTE, B. D. 1984. Forms of aqueous aluminum in acidified catchments of southern Ontario: a methodological analysis. Can. J. Fish. Aquat. Sci. 41: 766–776.

LAZERTE, B. D., AND P. J. DILLON. 1984. Relative importance of anthropogenic versus natural sources of acidity in lakes and streams of central Ontario. Can. J. Fish. Aquat. Sci. 41: 1664–1677.

LEGENDRE, L., AND P. LEGENDRE. 1983. Numerical Ecology. Elsevier Scientific Publishing Co., New York, NY.

MACKAY, R. J., AND K. E. KERSEY. 1985. A preliminary study of aquatic insect communities and leaf decomposition in acid streams near Dorset, Ontario. Hydrobiologia 122: 3–11.

MERRITT, R. W., AND K. E. CUMMINS, [ed.] 1984. An introduction to the Aquatic Insects of North America. Kendall/Hunt, 2nd ed. Publishing Co. Dobuque, IA. 722 p.

MINSHALL, G. W., AND P. V. WINGER. 1968. The effect of reduction in stream flow on invertebrate drift. Ecology. 49: 58–582.

MUNN, N. L., AND J. L. MEYER. 1988. Rapid flow through the sediments of a headwater stream in the Southern Appalachians. Freshwater Biol. 20: 235–240.

OLIVER, B. G., AND J. R. M. KELSO. 1983. A role for sediments in retarding the acidification of headwater lakes. Water Air Soil Pollut. 20: 379–389.

ORMEROD, S. J., P. BODE, C. P. MCCAHON, N. S. WEATHERLY, K. PASCOE, AND R. W. EDWARDS. 1987. Short-term experimental acidification of a Welsh stream: comparing the biological effects of hydrogen ions and aluminum. Freshwater Biol. 19: 341–356.

PETERSON, R. H., D. J. GORDON, AND D. J. JOHNSTON. 1985. Distribution of mayfly nymphs (*Insecta: Ephemeroptera*) in some streams of eastern Canada as related to stream pH. Can. Field-Nat. 99(4): 490–493.

PIELOU, E. C. 1975. Ecological Diversity. John Wiley and Sons, New York, NY.

REICE, S. R. 1985. Experimental disturbance and the maintenance of species diversity in a stream community. Oecologia. 67: 90–97.

REID, J. M., D. A. MACLEOD, AND M. S. CRESSER. 1981. Factors affecting the chemistry of precipitation and river water in an upland catchment. J. Hydrol. 50: 129–145.

RESH, V. H., A. V. BROWN, A. P. COVICH, M. E. GURTZ, H. W. LI, G. W. MINSHALL, S. R. REICE, A. L. SHELDON, J. B. WALLACE, AND A. WISSMAR. 1988. The role of disturbance theory in stream ecology. J. N. Am. Benthol. Soc.: 7: 433–455.

ROBINSON, C. T., AND G. W. MINSHALL. 1986. Effects of disturbance frequency on streams benthic community structure in relation to canopy cover and season. J. N. Am. Benthol. Soc. 5: 237–248.

ROWE, L., M. BERRILL, L. HOLLETT, AND R. J. HALL. 1989. The effects of short-term laboratory pH depressions on molting, mortality and major ion concentrations in the mayflies *Stenonema femoratum* and *Leptophlebia cupida*. Hydrobiologia. 184: 89–97.

SCHEIDER, W. A., D. S. JEFFRIES, AND P. J. DILLON. 1979. Effects of acidic precipitation on precambrian freshwaters in southern Ontario. J. Great Lakes. Res. 5: 45–51.

SHARPE, W. E., D. R. DEWALLE, R. T. LEIBFRIED, R. S. DINICOLA, W. G. KIMMEL, AND L. S. SHERWIN. 1984. Causes of acidification of four streams on Laurel Hill in southwestern Pennsylvania. J. Environ. Qual. 13(4): 619–631.

SILABEE, D. G., AND G. L. LARSON. 1982. Water quality of streams in the Great Smoky Mountain National Park. Hydrobiologia 89: 97–115.

SIMPSON, K., R. BODE, AND J. CALQUHOUN. 1985. The macroinvertebrate fauna of an acid-stressed headwater stream system in the Adirondack Mountains, New York. Freshwater Biol. 15: 671–681.

SCHLOSSER, I. J., AND K. K. EBEL. 1989. Effects of flow regime and cyprinid predation on a headwater stream. Ecol. Monogr. 59: 41–57.

SPRULES, W. M. 1947. An ecological investigation of stream insects in Algonquin Park, Ontario. Univ. Toronto Stud. Biol. Ser. 36, Publ. Ont. Fish. Res. Lab. 69: 1–81.

SPRULES, W. G. 1980. Nonmetric multidimensional scaling analyses of temporal variation in the structure of limnetic zooplankton communities. Hydrobiologia 69: 139–146.

STEWART, K. W. 1975. An improved elutriator for separating stream insects from stoney substrates. Trans. Am. Fish. Soc. 104: 821–823.

TIPPING, E., AND J. HOPWOOD. 1988. Estimating streamwater concentrations of aluminum released from streambeds during "Acid Episodes." Environ. Tech. Let. 9: 703–712.

WEATHERLEY, N. S., S. J. ORMEROD, S. P. THOMAS, AND R. W. EDWARDS. 1988. The response of macroinvertebrates to experimental episodes of low pH with different forms of aluminum, during a natural spate. Hydrobiologia 169: 225–232.

WIGGENS, G. B., AND R. J. MACKAY. 1978. Some relationships between systematics and trophic ecology in nearctic aquatic insects with special reference to Trichoptera. Ecology 59: 1211–1220.

WILKINSON, L. 1988. SYSTAT: the Systems for Statistics. Systat, Inc., Evanston, IL. 822 p.

LETTERS AND COMMENTS/ LETTRES ET COMMENTAIRES

Cycles in Lake Ecosystems

Reading "A Perspective on Great Lakes Fish Community Rehabilitation" (Christie et al. 1987) led C. S. Holling (University of Florida, Gainsville, FL 32611 USA, pers. comm.) to remark on the apparent absence of endogenous cycles of the terrestrial sort in aquatic systems. The question is interesting because it demands a close look at the whole issue of application of succession theory to aquatic ecosystems. The paper implicitly acknowledged that there is no aquatic analogue for rhythmic forest canopy opening (Pickett and White 1985; Bormann and Likens 1979). It suggested that enjuvenation in lakes must occur by way of more or less random flux in energy delivery to the primary production end, rather than through cyclic mortality driven by aging of the dominant organisms. The exceptions cited were the single year-class dominance of perch in oligotrophic Swedish lakes described by Alm (1951), and the "standing wave" effect in Keller Lake lake whitefish and lake trout (Johnson 1972), and both of these are clearly unusual for geographic, if no other, reasons.

The terrestrial effects of deforestation are so pervasive it is difficult to accept that the receiving waters could be regulated by production cycles which are independent of them. The effects of removal of forest cover on hydraulic regimes and nutrient transport to rivers and lakes are well understood (Bormann et al. 1974), but the nature of the linkage between terrestrial and aquatic production has apparently not yet been generalized. Autochthonous nutrient recovery from the bottoms of lakes is affected somewhat by annual energy input variations, but in general its year-to-year variance is not great. Annual allochthonous nutrient deliveries are relatively more important, but apart from episodic surges caused by tropical storms, floods and the like, the variations in annual loadings attributable to leaf fall and erosion are generally regarded as slight, and noncyclic.

One hypothesis that unifies land and water suggests that in undisturbed nature, cyclic canopy openings do affect nutrient and silt loadings to lakes lower on the watersheds, but the regularities in frequency and size of the openings tends to average out their effects. Since these are long cycles, the smaller the watershed, the more likely that cycles in lake productivity would parallel the terrestrial successional events.

The hypothesis can be tested by companion analyses of varving (pollen, carbon, etc) in drainage lake sediments and forest floor carbon horizons, in areas where terrestrial enjuvenation has been mainly caused by lightning fires.

The essential sterility of the debates about "bottom-up" vs. "top-down" control of lake system productivity which has occupied Great Lakes scientists recently lies with the reality that both effects operate in the same systems. The interesting questions concern the circumstances which create dominance for one or the other. There are situations in which nutrient delivery lags the energy available for its full utilization by the aquatic ecosystems, and equally, situations where nutrient materials arrive in quantities greater than the seasonal energetics can deal with. Even if the hypothesis proposed here is satisfactorily falsified, I suggest that information on the interactions of the energy and nutrient budgets of lakes produced by the study would provide considerable insight into the most basic question concerning lake ecosystem dynamics. At the more practical level, advances in the "Theory of Island Biogeography" (MacArthur and Wilson 1967) have improved capabilities for optimizing terrestrial faunal diversity in park planning and deforestation strategies, but so far these sorts of planning must proceed without reference to the aquatic components of the systems, and this represents a real deficiency in integrated resource management — W. J. Christie, *R.R. #4, Picton, Ont. K0K 2T0 Canada.* (JA584)

References

ALM, G. 1951. Year class fluctuations and span of life of perch. Inst. Freshwater Res. Drottningholm Rep. 33: 17–38.

BORMANN, F. H., AND G. E. LIKENS. 1979. Catastrophic disturbance and the steady state in northern hardwood forests. Am. Sci. 67: 660–669.

BORMANN, F. H., G. E. LIKENS, T. G. SICAMA, R. S. PIERCE, AND J. S. EATON. 1974. The export of nutrients, and recovery of stable conditions following deforestation at Hubbard Brook. Ecol. Monogr. 44: 255–277.

CHRISTIE, W. J., G. R. SPANGLER, K. H. LOFTUS, W. L. HARTMAN, P. J. COLBY, M. A. ROSS, AND D. R. TALHELM. 1987. A perspective on Great Lakes fish community rehabilitation. Can. J. Fish. Aquat. Sci. 44 (Suppl. 2): 486–499.

JOHNSON, L. 1972. Keller Lake: characteristics of a culturally unstressed community. J. Fish. Res. Board Can. 29: 731–740.

MACARTHUR, R. H., AND E. O. WILSON. 1967. The theory of island biogeography. Princeton University Press, Princeton, NJ. 203 p.

PICKETT, S. T. A., AND P. S. WHITE [ed.] 1985. The ecology of natural disturbances and patch dynamics. Academic Press, New York, NY. 472 p.

ANNOUNCEMENTS/AVIS

Announcement and Call for Papers

An International Symposium on the Production of Juvenile Atlantic Salmon in Natural Waters

The Department of Fisheries and Oceans is sponsoring a symposium in St. John's, Newfoundland, June 25–27, 1991, on the Production of Juvenile Atlantic Salmon in Natural Waters.

This international symposium will examine the ecology and population dynamics of young Atlantic salmon with a view to applications in estimating yields of smolts and assessing the status of the Atlantic salmon stocks.

Over the three days of the symposium there will be six sessions, the final one to be a synthesis. Other sessions will have the following themes:

- Habitat of juvenile salmon and the relationships of variables to productive capacity.
- Stream ecology; invertebrates; predators.
- Habitat improvement; competitive effects.
- Methods of evaluating standing stocks and the relationships with productive capacity of the habitat for juvenile salmon.
- Population dynamics and the relationships of juvenile salmon estimates to smolt yields.

Each session will have a keynote speaker of international reputation in his field.

Contributed presentations and posters are requested by the Program Committee. Titles and abstracts (limited to 200 words) are due December 1, 1990, and should be sent to: R. J. Gibson, Department of Fisheries and Oceans, P.O. Box 5667, St. John's, Nfld. A1C 5X1 Canada (Tel.: (709) 772-4466; Fax: (709) 772-2156/4347).

Selected papers will be published in a primary journal. Papers to be considered for publication must be submitted at the time of the symposium.

Annonce et demande de documents

Symposium international sur la production du saumon de l'Atlantique juvénile dans les eaux naturelles

Du 25 au 27 juin 1991, le Ministère des Pêches et des Océans parrainera à St. John's (Terre-Neuve) un symposium sur la production du saumon de l'Atlantique juvénile dans les eaux naturelles.

Ce sommet international examinera l'écologie et les dynamiques des populations de jeunes saumons de l'Atlantique afin de trouver un moyen d'estimer le rendement des smolts et d'évaluer l'état des stocks de saumon de l'Atlantique.

Au cours des trois jours de discussion, il y aura six séances, dont la dernière prendra la forme d'une synthèse. Les cinq autres séances porteront sur les thèmes suivants :

- L'habitat du saumon juvénile et les liens entre la productivité de l'habitat et les diverses variables.
- L'écologie des cours d'eau, les invertébrés et les prédateurs.
- L'amélioration de l'habitat et les effets de la concurrence.
- Les méthodes d'évaluation des stocks permanents et le rapport entre ces méthodes et la capacité de production de l'habitat.
- Les dynamiques des populations et les rapports entre les estimations de saumons juvéniles et le rendement des smolts.

À l'occasion de chacune de ces séances, un exposé sera présenté par un orateur de réputation internationale.

Le Comité d'organisation demande donc des affiches et des exposés pour ce symposium. Les titres et les résumés (d'au plus 200 mots) devront parvenir au plus tard le 1er décembre 1990 à : R. J. Gibson, Ministère des Pêches et des Océans, C.P. 5667, St. John's (T.-N.) A1C 5X1 Canada (Tél. : (709) 772-4466; Télécopieur : (709) 772-2156/4347).

Un certain nombre d'exposés seront choisis pour être publiés dans une revue principale. Les documents que l'on veut faire publier devront être présentés au moment du symposium.

Hydrobiologia

Editor-in-Chief

H.J. Dumont, *Ghent, Belgium*

Hydrobiologia publishes original articles in the fields of fundamental limnology and marine biology.
A wide range of papers are published, including ecology, physiology, biogeography, methodology and taxonomy. Applied (technological) papers are also published, provided they are of general interest and not solely technical in nature. Occasional very long papers and reviews are published at the invitation of the Editors, as are the proceedings of relevant, specialized symposia.

Subscription Information
ISSN 0018–8158
1990, Volumes 185–205 (63 issues)
Subscription rate: Dfl. 5691.00/
US$2656.50 *incl. postage/handling*

Subscribers also receive the issues of the **Journal of Applied Phycology** included in their subscription.

P.O. Box 322, 3300 AH Dordrecht, The Netherlands
P.O. Box 358, Accord Station, Hingham, MA 02018-0358, U.S.A.

Journal Highlight

KLUWER ACADEMIC PUBLISHERS

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 47, No. 11, November 1990

Volume 47, nº 11, novembre 1990

Ouellet, P., D. Lefaivre, and V. Koutitonsky. Distribution of shrimp (*Pandalus borealis*) larvae and hydrographic pattern in the northern Gulf of St. Lawrence 2068–2078

Shreffler, D. K., C. A. Simenstad, and R. M. Thom. Temporary residence by juvenile salmon in a restored estuarine wetland 2079–2084

Smith, C. L., and S. S. Hanna. Measuring fleet capacity and capacity utilization 2085–2091

Waples, R. S., and P. B. Aebersold. Treatment of data for duplicated gene loci in mixed-stock fishery analysis 2092–2098

Triska, F. J., J. H. Duff, and R. J. Avanzino. Influence of exchange flow between the channel and hyporheic zone on nitrate production in a small mountain stream 2099–2111

Lockhart, F. D., W. J. Lindberg, N. J. Blake, R. B. Erdman, H. M. Perry, and R. S. Waller. Distributional differences and population similarities for two deep-sea crabs (family Geryonidae) in the northeastern Gulf of Mexico 2112–2122

Carrasco, K. R., K. L. Tilbury, and M. S. Myers. Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects 2123–2136

Keller, A. E., and T. L. Crisman. Factors influencing fish assemblages and species richness in subtropical Florida lakes and a comparison with temperate lakes 2137–2146

Corkum, L. D. Intrabiome distributional patterns of lotic macroinvertebrate assemblages 2147–2157

Read, A. J., and D. E. Gaskin. Changes in growth and reproduction of harbour porpoises, *Phocoena phocoena*, from the Bay of Fundy 2158–2163

Fechhelm, R. G., and W. B. Griffiths. Effect of wind on the recruitment of Canadian Arctic cisco (*Coregonus autumnalis*) into the central Alaskan Beaufort Sea 2164–2171

Taylor, E. B. Variability in agonistic behaviour and salinity tolerance between and within two populations of juvenile chinook salmon, *Oncorhynchus tshawytscha*, with contrasting life histories 2172–2180

Holtby, L. B., B. C. Andersen, and R. K. Kadowaki. Importance of smolt size and early ocean growth to interannual variability in marine survival of coho salmon (*Oncorhynchus kisutch*) 2181–2194

Smith, B. D., G. A. McFarlane, and M. W. Saunders. Variation in Pacific hake (*Merluccius productus*) summer length-at-age near southern Vancouver Island and its relationship to fishing and oceanography 2195–2211

D'Amours, D., J. G. Landry, and T. C. Lambert. Growth of juvenile (0-group) Atlantic mackerel (*Scomber scombrus*) in the Gulf of St. Lawrence 2212–2218

Campana, S. E. How reliable are growth back-calculations based on otoliths? 2219–2227

McGeachy, S. M., and D. G. Dixon. Effect of temperature on the chronic toxicity of arsenate to rainbow trout (*Oncorhynchus mykiss*) 2228–2234

Millar, R. B. Comparison of methods for estimating mixed stock fishery composition 2235–2241

Ennis, G. P., R. G. Hooper, and D. M. Taylor. Changes in the composition of snow crab (*Chionoecetes opilio*) participating in the annual breeding migration in Bonne Bay, Newfoundland 2242–2249

Wood, C. C., and C. J. Foote. Genetic differences in the early development and growth of sympatric sockeye salmon and kokanee (*Oncorhynchus nerka*), and their hybrids 2250–2260

Hall, R. J. Relative importance of seasonal, short-term pH disturbances during discharge variation on a stream ecosystem 2261–2274

Letters and comments/Lettres et commentaires

Christie, W. J. Cycles in lake ecosystems 2275

Announcements/Avis 2276

© Minister of Supply and Services Canada 1990
Ministre des Approvisionnements et Services Canada 1990

CJFSDX47(11) 2068–2276 (1990)
ISSN 0706-652X

Subscription rates/Abonnements

Canada: Institutional/Collectif $200; Personal/Personnel $75
Other countries/Autres pays : Institutional/Collectif $240; Personal/Personnel $90
Single issue/Par numéro : $13.95 (Canada); $16.75 (Other countries/Autres pays)